

# Intrauterine fetal death: classification and risk factors

A case-control study of sociodemographic, clinical and thrombophilic risk factors



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## Abbreviations

aCL	anticardiolipin antibodies
AHUS	Akershus University Hospital
APTT	activated partial thromboplastin time
anti- $\beta_2$ GP1	anti- $\beta_2$ glycoprotein 1 antibodies
AT	antithrombin
APO	adverse pregnancy outcome
CI	confidence interval
CODAC	Causes Of Death and Associated Conditions - classification for perinatal deaths
CRF	case-report-form
EDTA	ethylene-diamine-tetra-acetic-acid
ELISA	enzyme-linked immunosorbent assay
FV Leiden	factor V Leiden polymorphism (FV rs6025)
HD	hypertensive disorders
HT	hypertension
IUFD	intrauterine fetal death
ICD	International Classification of diseases
ICSI	intracytoplasmic sperm injection
IgG	immunoglobulin type G
IgM	immunoglobulin type M
IUGR	intrauterine growth restriction
IVF	in vitro fertilization
LA	lupus anticoagulant
LR	lupus ratio
MBR	the Norwegian Medical Birth Registry

OUH	Oslo University Hospital
PAR	population attributable risk
PC	protein C
PCR	polymerase chain reaction
PE	preeclampsia
Prothrombin polymorphism	prothrombin gene G20210A polymorphism (F2 rs179963)
PS	protein S
PSANZ-PDC	Perinatal Society of Australia and New Zealand – Perinatal Death Classification
ReCoDe	Relevant Condition at Death
RR	relative risk
RVV	Russell viper venom
RVVT	Russell viper venom time
SGA	small for gestational age
VIP	Venous Thromboembolism In Pregnancy
VTE	venous thromboembolism
WHO	World Health Organization



## Summary

**Background:** Stillbirth or intrauterine fetal death (IUFD) is a severe and difficult event for the parents and occurs in approximately five of 1000 births in high-income countries. Understanding of causes and recognition of risk factors is essential for preventive measures, counseling of parents, surveillance of health care and comparison both nationally and internationally.

**Objectives:** To estimate the incidence of stillbirths in a Norwegian population, classify according to cause of death, investigate socio-demographic, clinical and thrombophilic risk factors and to evaluate variations in risk estimates by different control selection.

**Methods:** 377 women with IUFD after 22 gestational weeks at two university hospitals in the Oslo area, in the period 1990 to 2003, were classified according to the Cause Of Death and Associated Conditions (CODAC) classification of perinatal deaths. They were compared with two different control-groups for the identification of socio-demographic and clinical risk factors. A subsample of 105 cases and 262 controls comprised the study population for acquired and inherited thrombophilic risk factors.

**Results:** The incidence of stillbirths was 4.1 per 1000 deliveries. The majority (68%) had placental pathology as a main cause of death or as an associated condition. Small for gestational age (SGA) and placental abruption were strongly associated with IUFD, but hypertensive disorders were moderate risk factors if not mediated through SGA. Other risk factors were of low prevalence and of limited importance in the prevention of IUFD. Risk factors differed according to cause, apart from smoking and SGA that were risk factors in all causal groups. Lupus anticoagulant was associated with a history of IUFD, although probably confined to women with multiple positivity for antiphospholipid antibodies. The prothrombin gene G20210A polymorphism was also associated with IUFD, most prominent in the group of placental causes.

## **List of papers**

### **Paper I**

Helgadóttir LB, Skjeldestad FE, Jacobsen AF, Sandset PM, Jacobsen EM. Incidence and risk factors of fetal death in Norway: a case-control study. *Acta Obstet Gynecol Scand* 2011; 90:390-7.

### **Paper II**

Helgadóttir LB, Skjeldestad FE, Jacobsen AF, Sandset PM, Jacobsen EM. The association of antiphospholipid antibodies with intrauterine fetal death: a case-control study. *Thromb Res* 2011. In press.

### **Paper III**

Helgadóttir LB, Skjeldestad FE, Jacobsen AF, Sandset PM, Jacobsen EM. The association of inherited thrombophilia and intrauterine fetal death: a case-control study. *Blood Coagul Fibrinolysis* 2011. In press.

### **Paper IV**

Helgadóttir LB, Turowski G, Skjeldestad FE, Jacobsen AF, Sandset PM, Roald B, Jacobsen EM. Classification of stillbirths by cause of death and risk factor analysis - an observational case-control Study. Submitted for publication.

## **1. Introduction**

In high-income countries the population in general expects that every pregnancy will lead to the birth of a healthy baby. The perception is that stillbirths are something of the past. However, although the rates of stillbirths have fallen remarkably over the past 50-60 years, they are still not rare events and are devastating for the parents. Identification of causes and risk factors is necessary for stillbirth prevention. Scandinavian studies on stillbirth ((intrauterine fetal death (IUFD))), have most often focused on a single or limited number of risk factors and the studies addressing multiple risk factors have reported on either unexplained IUFD only [1], or on perinatal mortality [2,3] and not on stillbirths in general. This accounts also for available epidemiological data from Norway.

Classifying stillbirths according to cause is needed for the purpose of prevention, counseling and comparison. In addition risk factors may vary between different causal groups. However, assigning a single cause of death is challenging and the use of suboptimal classification systems may contribute to a higher proportion of unexplained deaths.

Previous to this study there were no data from Norway on the association of acquired or inherited thrombophilia and IUFD. Studies investigating the association between inherited thrombophilias or antiphospholipid antibodies (APAs) and IUFD have often been of small sample size [4-6], and they have differed in selection criteria for cases and controls [7,8]. In addition, investigators reporting on the association between APAs and IUFD have usually analyzed the prevalence of APAs in blood samples collected within months after suffering IUFD. To our knowledge, the prevalence of APAs several years after the incident, among women with a history of IUFD, has not been reported.



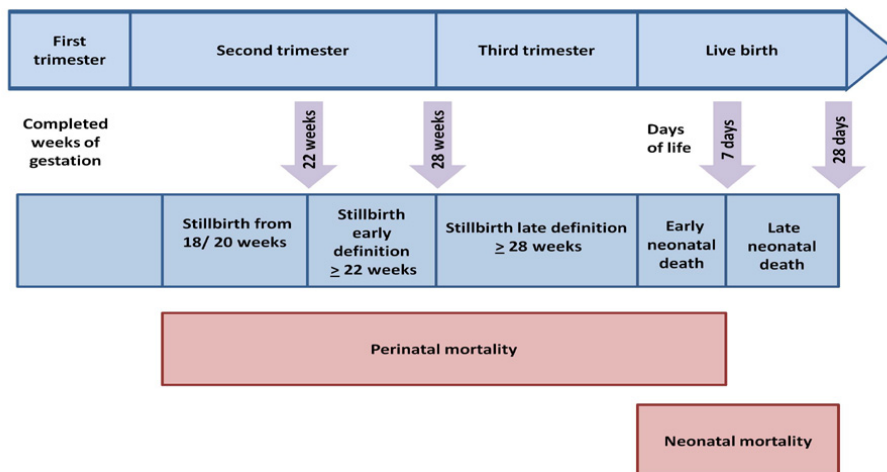
## **2. Background**

### **2.1 Stillbirth - intrauterine fetal death (IUFD)**

#### **2.1.1 Definition**

Inconsistent use of definitions and terminology has contributed to confusion about stillbirths or IUFDs (I will use the fraises/terms interchangeably in the thesis). In addition to changes in definitions over time, there are great variations in the terminology between countries, with greater variability between high-income countries than between low-income countries [9,10] (Figure 2.1). In USA alone nine different definitions have been reported [11]. The gestational age by which stillbirth is defined varies from 18 to 28 weeks [12]. The WHO definition of stillbirth refers to the birth of an infant weighing at least 500 g, or born at 22 or more completed weeks' of gestation, or with a crown-to-heel length of 25 cm or more [13]. In this definition birth weight takes priority over gestational age since birth weight is thought to be more reliably reported, even though in low-income countries many stillborn infants are never weighed [14,15]. However, in many instances the use of the gestational age is preferred rather than birthweight, especially in high-income countries where ultrasound timing of pregnancy is standard practice. This leads to higher reported stillbirth rates; as an example, if gestational age ( $\geq 22$  weeks) is used rather than birthweight ( $\geq 500$  g) in Norway, the reported stillbirth rate is 15% higher [16]. For international comparison, WHO recommends reporting stillbirths in the third trimester, meaning stillbirths of infants weighing 1000 g or more, born at 28 or more completed weeks' of gestation, or with a crown-to-heel length of 35 cm or more [13,17]. Perinatal mortality is a wider term and includes neonatal deaths in the first week of life in addition to stillbirths. Neonatal mortality refers to the death of a live born infant within the first 28 days of life (Figure 2.1).

**Figure 2.1.** Terminology. modified from Lawn JE et al. 2011 [18] with permission.



### 2.1.2 Incidence

Counting the numbers and registering causes of stillbirths is essential in any approach to prevention. Stillbirth is not a rare incident and each day more than 7300 babies are stillborn on a worldwide basis ([www.thelancet.com/series/stillbirth](http://www.thelancet.com/series/stillbirth) (from the executive summary for the series)). If stillbirths were aligned with the leading global causes of death in all categories, they would rank fifth among the global health burdens [19,20]. In 2009, the worldwide estimated third trimester stillbirth rate was 18.9 stillbirths per 1000 births, declining from 22.1 per 1000 births in 1995 [21]. The highest estimated rate of 47 per 1000 births was in Pakistan and the lowest in Finland (2.0 per 1000 births). In 2008 all the Nordic countries had third trimester stillbirth rates in the range 2.0-2.7 per 1000 births [21]. The incidence of IUFD in developed countries varies from approximately 2 – 7.5 per 1000 pregnancies [14,21-23]. Currently stillbirths account for more than 50% of all perinatal deaths in developed countries [22,24]. For comparison, stillbirths are 10 times more common than the sudden infant death syndrome (SIDS) [25].

The estimated number of global third trimester stillbirths ( $\geq 28$  weeks) was 2.64 million (uncertainty range 2.14 - 3.82 million) in 2009, 76.2% occurring in South Asia and Sub-Saharan Africa [14,21]. These are the estimated numbers, but probably another 1 – 2 million stillbirths occur, that are not reported [26]. Stillbirths occurring before 28 gestational weeks are rarely reported in low-income countries [16], and many other countries do not estimate their numbers, even though these can represent one third of all stillbirths in high-income countries [27]. Of all stillbirths, 98% occur in low- or middle-income countries, and low-income countries are now where high income countries were 50-100 years ago in terms of stillbirth rates [9,28]. In high-income countries, some ethnic and lower income groups have higher stillbirth rates than the national average [18]. Such variations in stillbirth rates within the same country are reported to be closely associated with social deprivation, poor maternal health and availability and quality of health services [29] and are probably a sensitive marker of inequity [30]. The overwhelming preponderance of stillbirths in low- and middle-income countries can be explained by poor obstetric care as well as higher prevalence of risk factors, especially nutritional factors and birth spacing [31]. Intrapartum stillbirths, viable with better obstetric/intrapartum care [32], account for almost half of all stillbirths [18] in low-income countries, but only rarely occur in developed countries (less than 0.5 per 1000 total births) [18]. Intrapartum stillbirth rates have been proposed as a measure of quality of intrapartum care [33].

In high-income countries approximately 5 per thousand pregnancies, that reach 22 gestational weeks, result in stillbirth [34], or if using the international comparison limit of 28 gestational weeks, almost four per 1000 of all births [21]. The rate of perinatal deaths has declined markedly over the past 50-60 years [35], to a large extent on account of changes in obstetrical practice and antenatal care. Several strategies have contributed to this. Firstly, elimination of risk factors with better control of diabetes and hypertensive disorders and

reduction in maternal smoking. Secondly, prevention of stillbirth by antenatal monitoring with non-stress tests, biological profile or Doppler examinations and correct timing of induction of labor. Thirdly, intrapartum monitoring and finally, antepartum screening for fetal anomalies [36]. However, while neonatal deaths have been steadily decreasing the last 20-30 years, the stillbirth rates have been relatively stable [9]. It seems that there has been some reduction in late ( $\geq 28$  weeks), but hardly in early stillbirths [27,37]. There are even reports of increased incidence of stillbirths the last decade [25].

### **2.1.3 Causes**

Targeting specific causes and specific clinical scenarios is crucial for further prevention of stillbirths in high-income countries. However, the determination of a cause can be challenging, since the circumstances of the death can be complex and thus the value of a thorough investigation must be emphasized. The most common causes of stillbirth worldwide are: complications of childbirth, maternal infections in pregnancy, maternal disorders, fetal growth restriction and congenital abnormalities [18]. Causes as well as risk factors differ between low- and high-income countries [38] and these differences correlate with the stillbirth rates [18]. As an example the proportion of intrapartum stillbirths is higher in countries with higher stillbirth rates. But the causes differ not only because of their true prevalence, but also because of different potentialities in identifying the causes [16].

Causes of stillbirths include congenital anomalies, infections, asphyxia, placental abruption and umbilical cord accidents. In developed countries 25% of stillbirths have an intrinsic fetal cause, 6-12% are caused by or associated with a major chromosomal abnormality and a number of autosomal recessive metabolic disorders are known to result in stillbirth [39]. Maternal and/or fetal infections probably cause 10-25% of stillbirths, most often in the early



preterm period (<28 weeks of pregnancy) [40,41]. The most important infectious agents reported in high-income settings are parvovirus B19, group B streptococci, toxoplasma gondii, Listeria species, E. coli, enteroviruses, cytomegalovirus (CMV), and influenza virus [42]. Some pathogens like parvovirus B19, CMV and toxoplasma have a clear causal relationship with IUFD, while others are associated with an increased risk of stillbirth, with strong evidence of a causal relationship absent (colonisation with ureaplasma urealyticum, mycoplasma and group B streptococci) [38,40,41].

Umbilical cord accidents may cause 15% of stillbirths [43], but cord incidents are also common in live births, so this diagnosis should be made with caution. Placental abruption accounts for 10 – 20% of all intrauterine fetal death, but occurs in only 1% of pregnancies [44]. Feto-maternal hemorrhage (other than placental abruption) is probably underestimated as a cause of stillbirth, but may contribute to 5% of stillbirths [45]. About 10% of fetal deaths can be related to maternal medical illnesses such as hypertension, diabetes mellitus, systemic lupus erythematosus, chronic renal disease, thyroid disorders and cholestasis in pregnancy [46], but stillbirths caused by these disorders have been greatly reduced in numbers the last decades thanks to better management and care.

Causes of stillbirths vary according to gestational age. Fretts et al. reported that the most common causes in weeks 24-27 were infections (19%), placental abruption (14%) and fetal anomalies (14%), with 21% unexplained, while after 28 gestational weeks unexplained stillbirth was the largest group (26-40%), with fetal malnutrition (14-19%) and placental abruption (12-18%) being frequent as well [23,47]. The prevention of early fetal losses has been the most difficult to achieve.

#### **2.1.3.1 Unexplained stillbirth**

Unexplained stillbirth is a death unexplained by fetal, placental, maternal or obstetric factors.

“Unexplained stillbirth” is not synonymous with “stillbirths of unknown cause”, since the cause can be unknown because of lack of information or suboptimal examination and/or classification. Approximately 25% of stillbirths are reported to be unexplained [1,48], with numbers ranging from 9 – 71% [49-51]. The proportion of unexplained stillbirths varies according to the classification system used. Classification according to the Wigglesworth and Aberdeen classifications result in a large proportion of unexplained stillbirths [52,53], and such classification systems do not seem to adequately fulfill their purpose. The proportion of unknown or unexplained stillbirths is also dependent on what information is available and is higher when information is scarce. The last decades, the overall rate of unexplained stillbirths has declined by approximately 65%, but the proportion of unexplained stillbirths among all stillbirths has remained relatively constant, or has even increased [54-56].

The proportion of unexplained stillbirths increases with advanced gestational age, with half of them occurring after 38 gestational weeks [1,47,55,57], more pronounced in women of advanced maternal age [54]. Huang et al. described risk factors in a large study of unexplained stillbirth. These included: advanced maternal age, low education, small-for-gestational-age (SGA), large infants, primiparity, parity  $\geq 3$ , and the presence of cord loops [55]. Frøen et al. reported similar findings in Norway for sudden intrauterine unexplained death (SIUD), and pre-pregnancy obesity and body mass index (BMI)  $> 25 \text{ kg/m}^2$  in addition [1]. Smoking is also a well-known risk factor for unexplained stillbirth of growth restricted infants [48,58].

#### **2.1.4 Classification of IUFD**

Classification of stillbirths is needed for the purpose of prevention, counseling, quality improvement, comparison, surveillance of health care and research both nationally and internationally. It should help clinicians to understand what went wrong to derive learning points

for best clinical practice. However, assigning a single cause of death can be challenging due to the complexity of the clinical situation within which the fetus dies [59]. The purpose of classification systems is information management: capture, storage and retrieval [60]. Therefore, ideally classification systems for stillbirths should be able to capture clinical entities, besides the direct cause of death, since this can be important for a deeper insight into the case and for management and counselling. The use of suboptimal classification systems may lead to loss of important information and contribute to a higher proportion of unexplained deaths.

Classification of IUFDs is complex as a result of the interaction between pathophysiological processes in the mother, fetus and placenta. Different definitions, different routines in investigating and different methods of classifying stillbirths impede comparison of causes and risk factors. Stillbirths can be classified in many dimensions: 1) gestational age at birth; early stillbirths (20-28 weeks) and late stillbirths (after 28 weeks) [38], 2) according to the onset of labor; before (antepartum) or after (intrapartum) the onset of labor, and 3) cause, which can be identified from different points of view: pathophysiological pathways, maternal conditions, obstetrical complications, fetal conditions or mechanism of death.

Categorizing stillbirths by cause is important for targeting preventive strategies. On a worldwide basis this is difficult, because of limited stillbirth data in low-income countries and the lack of classification systems compatible for use in these countries. A good classification system should be useful in all countries and for all populations. Good stillbirth data are available in some high-income countries through national perinatal surveillance systems, although even within Europe there can be paucity of comparable stillbirth data [61].

Since 1954 more than 30 different classification systems have been in use [62], but multiple classification systems with poor comparability impede international comparison of the causes of IUFD. A single system universally accepted would make classification easier and

facilitate international comparison. However, such a system does not exist, apart from the International Classification of Diseases (ICD) 10, which is not adapted specifically to stillbirths and does not fully recognize the stillborn infant [16]. The extended Wigglesworth [52] and modified Aberdeen [63] have been the most widely used classification systems throughout the world [53]. Classification systems are made with different purposes and different perspectives/emphasis, but each will provide the results it was designed for. Following are some examples of the various approaches and classification systems for stillbirth classification:

1) An obstetric approach in clinico-pathological classifications (developed by obstetricians), tries to identify why the infant died, analyzing the obstetric factors that lie behind the death, in addition to congenital anomaly, isoimmunization, maternal disorders and special fetal conditions. Here under are; a) the first approach to classification of perinatal deaths by Baird et al, called the Aberdeen classification [64], b) the modified version of the Aberdeen classification by Cole et al. [63] and c) the Whitfield classification, which is based on the other two, including more detailed information [65].

2) Systems based on the pathophysiological entity initiating the chain of events that leads to death; a) the Tulip classification based on clinical and pathological findings for the purpose of counseling and prevention (includes also information on the mechanism of death) [62], b) Perinatal Society of Australia and New Zealand – Perinatal Death Classification (PSANZ-PDC) [66] and c) the Causes Of Death and Associated Conditions (CODAC) classification for perinatal deaths [60].

3) Systems concentrating on the mechanisms of death; the Tulip classification [62].

4) Systems concentrating on fetal factors or the clinico-pathological processes within the infant; a) a classification based on autopsy findings by Bound et al. [67], b) a simpler classification, based on externally observable features, ascertained by the history – the

Wigglesworth classification [59], and c) a more detailed classification by Hey et al. based on the original one by Bound et al. [68].

**5)** Classification of stillbirth by relevant condition at death (ReCoDe) [50]. A system that seeks to identify the relevant condition at the time of death in utero, in the mother, fetus or placenta. It seeks to establish what went wrong, either the cause of death and/or other relevant conditions, not necessarily why. More than one category can be coded.

**6)** Systems capturing associated conditions and risk factors in addition to the cause of death; a) PSANZ-PDC [66] and b) CODAC [60].

Some classifications include clinical conditions like hypertensive disorders and intrauterine growth restriction (IUGR) as causal groups [50,63,65,66], in contrast to others that claim that these clinical factors are manifestations, symptoms, of a pathophysiological entity, and should rather be recorded as associated conditions, or as risk factors, as in CODAC. Many systems have a hierarchical structure, one cause “winning” over another in a systematic way [50,59,63]. This however forces one to choose or designate only one cause for each case, possibly losing important information on contributing factors.

Simple systems deficient in subgroups can be too crude and valuable information may be lost [69]. Early classification systems, like the Wigglesworth [59], and Aberdeen classifications [64] included only few basic groups, and although newer modified versions [52,63] include possibilities for a more detailed classification they are still quite simple. Some newer classifications have attempted to obtain more information but are often mainly designed for countries where thorough investigation including laboratory analyses, autopsy of the infant and pathological examination of the placenta is possible [62] and are impractical when data is scarce and the only information available often through verbal autopsy (interview with the mother or caregiver) occurring a year or later after the loss.

The explained proportion of stillbirths varies according to which classification system is used and with the level of investigation [51,70,71]. Information on maternal and fetal health, the placenta and autopsy are the most important sources of information [51]. Many maternal conditions and characteristics are potentially associated with stillbirths, indicating the importance of collecting data on maternal conditions as well. Several reports have indicated the importance of placental pathology as a source of information in investigating the causes of stillbirths [51,53,72], but only the Tulip [62] and CODAC [60] classification systems include detailed categories for this purpose.

#### ***2.1.4.1 CODAC classification of perinatal deaths***

The CODAC classification system is designed to retain information on the main cause of death as well as up to two associated conditions [60]. It is a classification system for perinatal deaths, recently developed by an international group of investigators. It was developed with a basis in some fundamental elements classification systems should incorporate [60]:

- 1) Compatibility with the ICD.
- 2) Expandability of classifications. Expandable main categories, when detailed information is available, but possible to use the main groups when information is limited.
- 3) Capture of intrapartum events.
- 4) Capture of placental conditions.
- 5) Ability to differentiate unknown and unexplained events.

CODAC has been evaluated and compared with 5 other classification systems [51] and received the highest score regarding the ability to retain important information and the ease of use, lowest proportion of unexplained stillbirths and a fair inter-observer agreement.

The main focus of CODAC is the “cause of death” (COD), with the possibility of coding for up to two additional “associated conditions” (ACs) to preserve more detailed information

**Table 2.1.** The CODAC classification system. Cause of death (COD).

CODAC – COD Main groups Level I		CODAC – COD Subgroups Level II 0: always unspecified or other	CODAC - COD Level III Sub-sub groups 0: always unspecified 9: always other (free text)
0	Infection	1 HIV 2 Malaria 5 GBS 6 Common bacteria of maternal flora – non-GBS 7 Bacteria – other 9 Viral - other	<p>Example: The 3-digit COD <b>71?</b></p> <p>Level I: 7 - Maternal Level II: 1 - Hypertensive Disorders</p> <p>Level III: 0 Unspecified 1 Eclampsia 2 HELLP syndrome with or without eclampsia 3 Hypertension / Hypertensive crisis - unspecified 4 Hypertension / hypertensive crisis in pregnancy-induced HT/ Preeclampsia 5 Hypertension / hypertensive crisis in pre-existing HT 6 Ehler-Danlos syndrome 7 Pulmonary hypertension - other 9 Other</p>
1	Neonatal		
2	Intrapartum	1 Uterine rupture 2 Cord and placenta complications 5 Prolonged/ obstructed and incomplete labor 6 Extreme prematurity 9 Unknown (fetal respiratory failure/ asphyxia)	
3	Congenital anomaly	1 Central nervous system 2 Cardiovascular and lymphatic vessels 7 Trisomies	
4	Fetal	2 Brain injury 2 Cardiac 3 Alloimmunization 7 Hydrops of unknown origin 9 Infection / inflammation of the fetus	
5	Cord	1 Knots 2 Loops 3 Abnormal insertion 4 Focal anomaly 5 Generalized anomaly 6 Other mechanical compromise 7 Thrombosis of the cord 9 Infection / Inflammation of the cord/ vessels	
6	Placenta	1 Abnormal implantation, migration or shape 2 Villous / vascular maldevelopment 3 Abruptio or retroplacental hematoma 4 Infarctions and thrombi 5 Circulatory disorder 7 Transfusion and feto-maternal hemorrhage 8 Small for gestation placenta 9 Infection / inflammation of the placenta/ membranes	
7	Maternal	1 Hypertensive disorder 3 Diabetes 5 Hematology 8 Trauma 9 Infection	
8	Unknown	1 Unknown with no placental PAD nor autopsy 2 Unknown with no placental PAD 3 Unknown with no autopsy 5 Unknown despite autopsy and placental PAD 6 Unexplained despite full evaluation	
9	Termination		

The table contains the groups possible for the main (single) or secondary COD: all Level I groups (10), the Level II groups of relevance to our study (44 of 94) and column 4 comprises an example of a 3-digit COD with the possible Level III categories (9 of 577).

about the case. By this it is possible to capture the narrative of the case within the system. For classification in the main groups, a skilled birth attendant can easily observe the information/details needed, thus the system is applicable also in low-resource settings.

For each case, CODAC allows up to three codes with three digits each (123 123 123), although only one code (123) is necessary. The first (or single) code represents the main COD. The second code can represent a secondary COD (if the first is not thought to be sufficient to fulfill the criteria of a solitary COD) or an AC, and the third code represents an AC. The first digit in each code represents “Level I” or the main categories of the COD or the AC (Tables 2.1 and 2.2). The second and third digits, in each code, represent Levels II and III, each level

**Table 2.2.** The CODAC classification system. Associated conditions (AC).

CODAC – AC Main groups Level I		CODAC – AC Subgroups Level II Groups 0-7: same subgroups as for COD	CODAC – AC Sub-subgroups Level III 0: always unspecified 9: always other
0	Infection		
1	Neonatal		
2	Intrapartum		
3	Congenital anomaly		
4	Fetal		
5	Cord		
6	Placenta		
7	Maternal		
8	Associated perinatal	0 Other or unspecified 1 Small for gestational age 2 Macrosomia 3 Multiples 4 Amniotic fluid 5 Assisted reproductive technique 6 PPROM 7 Post-term pregnancy 8 Vaginal hemorrhage 9 Sub-optimal care	Example: The 3-digit AC <b>87?</b>  Level I: 8 -Associated Perinatal Level:II: 7 - Post-term pregnancy  Level III: 0 Unspecified 1 > 41 completed weeks 2 > 42 completed weeks 3 > 43 completed weeks 9 Other
9	Associated maternal	0 Other or unspecified 1 Obstetric history 2 Smoking 3 Recreational and addictive drugs 4 Medication – adverse effects 5 Poverty 6 Maternal characteristics	

The table contains the groups possible for an AC: all Level I groups (10), the Level II groups of main groups 8 and 9 (17 of 94) and column 4 comprises an example of a 3-digit AC with possible level III categories (5 of 577).



representing more detailed information. Each Level I category is comprised of several Level II categories which in turn are comprised of several Level III categories. Stillbirth caused by placental abruption could have a main (or single) COD **630** (Level I - placenta, Level II- abruption or retroplacental hematoma, Level III – unspecified), but if the cause was placenta infarctions the COD would be **640** (Table 2.1). To be a COD the condition should be expected to be mortal in a significant proportion of cases (5%) and to be an AC the condition should contribute significantly in explaining the circumstances of death. An AC can never fulfill the criteria of a COD. The system includes ten main groups (Level I), 94 sub-groups (Level II) and 577 sub-sub-groups (Level III).

The order of codes, both COD and AC, should preserve the relative significance and sequence of events, the most relevant code first. Hierarchy is only used when information on sequence and significance is lacking. The decision of which COD or ACs are assigned in each case is a subjective expert opinion, after review of all available information. For support ten coding rules have been defined [60] and for consistency in coding these rules need to be followed.

Each case is thus necessarily represented by at least one 3-digit code, but can be represented by as much as a three 3-digit codes. This enables reporting for each case by groups of disorders rather than attributing the death to a single event or disorder. In this way CODAC is well equipped to capture and retain information, as was demonstrated in an assessment by the International Stillbirth Alliance [51]. In order to classify the case in CODAC only minimum of information is mandatory, but a thorough investigation and more detailed information allows more accurate classification. This wide frame makes CODAC suitable in different settings.

### **2.1.5 Risk factors**

#### *Definition*

A risk factor is a maternal characteristic associated with and increasing the likelihood of stillbirth, but without a known causal pathway leading to death and can be present in many cases of live births as well [36].

Fortunately the majority of pregnant women are at low-risk and for them the risk of a late stillbirth is relatively low (1-2 per 1000 pregnancies) [73]. However, identification of risk factors is essential for the purpose of better care and preventive measures to decrease the likelihood of stillbirth. This is the purpose of good antenatal follow-up and obstetrical practice. Changes in obstetrical practice, like the improved management of diabetes, pre-eclampsia and Rhesus-isoimmunisation, are probably responsible for the declining stillbirth rates the last decades [73].

#### **2.1.5.1 Sociodemographic risk factors**

##### **Maternal age**

Large epidemiologic studies have reported advanced maternal age to be associated with increased risk of stillbirth, not explained by age-related risk for pregnancy related complications such as pre-eclampsia, gestational diabetes, multiple pregnancy or placental abruption [1,23,74-77]. The reported ORs for the risk of IUFD associated with advanced maternal age are in the range 1.3-1.9 for age 35-39 years and 1.7-3.3 for age over 40 years. Frøen et al. reported an OR of 5.1 (95% CI 1.3-19.6) for the risk of unexplained intrauterine death among women 35 years and older [1]. This maternal age-related risk of stillbirth has been described to increase with advanced gestational age and is specially associated with unexplained stillbirth [54,75,76,78]. The maternal age-related risk of stillbirth is quite important since the obstetrical population is changing in developed countries and the number of births to women 35 years and older is increasing. Interestingly Fretts et al. found that the relative risk of stillbirth for women of

advanced maternal age ( $> 35$  years) had increased since the 1960's [75]. An association between young age and stillbirth has also been reported, although an inconsistent finding. Bateman et al. found women  $\leq 19$  years being more likely to have a pregnancy outcome of stillbirth, compared to women 20-34 years old, with OR 1.11 (95% CI 1.08-1.14) [76]. Olausson et al. investigated the association of young maternal age and perinatal death. The rates of stillbirth  $\geq 28$  weeks increased gradually with lower maternal age, although not statistically significant when comparing women at ages  $< 19$  years to women 20-24 years of age [79], but low maternal age was significantly associated with neonatal death.

### **Maternal weight / BMI**

Pre-pregnancy obesity or increased BMI is associated with an increased risk of stillbirth [80-83], with ORs from individual studies in the range 1.9-2.7 for overweight (BMI 25-29.9 kg/m<sup>2</sup>), and 2.1-2.8 for obese women (BMI  $\geq 30$ ) [23]. A recent meta-analysis found the odds of stillbirth to be increased 23% (OR 1.23; 95% CI 1.09-1.38) and 63% (OR 1.63, 95% CI 1.35-1.95) for overweight and obese women, respectively [34]. This poses a growing problem since the prevalence of overweight and obesity is increasing in most high-income countries. In USA 54% of women aged 20-39 are either overweight or obese [84] and Europe seems to follow a similar pattern albeit with some delay [85]. In fact overweight and obesity are reported to be the most prevalent risk factors for stillbirth in high-income countries [34]. This association appears to be strongest among nulliparous women [82] and seems to increase with advanced gestational age [81].

The risk of stillbirth associated with overweight or obesity can be mediated through other factors as these women have increased risk for pregnancy complications such as gestational diabetes and pre-eclampsia and they are also more likely to smoke and to have a low socio-economic status [86-88]. However, overweight/obesity remains as an independent risk factor

even after adjusting for other known risk factors [81]. It is possible that obese women without clinical disease may present with metabolic and vascular abnormalities similar to those seen in pre-eclamptic women with failure of normal placentation [87,88]. A large Danish cohort study reported increased risk for stillbirth among obese women, gradually increasing with advancing gestational age with a 340% risk after 40 weeks. They showed a similar trend among overweight women with doubled risk after 40 weeks. The overweight or obese women had more often obesity associated diseases such as hypertensive disorders and diabetes mellitus, but excluding those women from analysis only moderately affected the risk of stillbirth. The stillbirths among the obese women (without obesity related diseases) were more likely to be caused by placental dysfunction or to be unexplained [89].

### **Parity**

Both primiparity [77] and multiparity [75] have been found to be associated with stillbirth [55], but these are not consistent findings [75,90,91]. The recently published meta-analysis of Flenady et al. found a 42% increase in the odds of stillbirth associated with primiparity (OR 1.42; 95% CI 1.33-1.51) [34]. The number of primiparous women of advanced maternal age (>35 years) is rising in high-income countries because of delayed childbearing and studies have demonstrated that this sub-group of older primiparous women has a greater risk of stillbirth than young primiparous women [81].

### **Socio-economic factors**

Low socio-economic status has been associated with increased risk of stillbirth [34,55,92]. Socioeconomic status can be measured by several factors like: education, employment, income and marital status. Several studies have found single civil status to be associated with stillbirth, probably as a factor of lower socio-economic status as a single mother [48,49,75,78,90]. In the Nordic countries an association between social differences and stillbirth is reported, with a

relative risk in the range 1.4-1.9 for the groups with greatest deprivation [93]. Reports from other western countries show similar figures [94]. Smoking is probably a great contributor to stillbirths in some disadvantaged populations [34], and these women also have increased prevalence of other risk factors for stillbirth, like overweight. However, the increased stillbirth risk cannot entirely be explained by that, as adjusting for overweight, smoking and several other risk factors only slightly changes the risk estimates [92]. It has been suggested that the elevated stillbirth risk among these women might be caused by subtle differences in care [24].

### **Suboptimal care**

In high-income countries suboptimal care can include: delayed diagnosis of relevant pregnancy complications or delayed or inadequate reactions of health care providers. The pregnant women contribute by factors like inadequate antenatal attendance and smoking. Suboptimal care is reported to affect the stillbirth risk, and is shown to be associated with 10-60% of stillbirths and neonatal deaths in high-income countries [27]. One study found that not attending antenatal care was associated with a three-fold increase in the odds of stillbirth [95]. The difference in stillbirth risk between indigenous and non-indigenous women in Australia might also partly be explained by a larger proportion of indigenous women living in rural areas of socioeconomic deprivation with limited access to antenatal care [96]. In the USA black women have been found to be four times more likely to have no prenatal care and this combination of no prenatal care and black race has been associated with a seven-fold risk of stillbirth [95,97].

### **Ethnic origin**

Racial disparity of stillbirth risk has been reported in several studies [76,90,91,98]. The stillbirth rate among non-Hispanic black women is reported to be more than double the rate of non-Hispanic white women in USA [98], and indigenous Australian women have almost twice the risk of non-indigenous women [96]. Sharma et al. also found greater risk of recurrence of

stillbirth among African American women compared to white women, OR 2,6 (95% CI 1.2 - 5.7) [91]. Herschel et al. found that black women had a nine-fold increase in hypertensive associated fetal mortality, two-fold increase in abruption and a larger proportion of deaths associated with SGA [99].

The higher rate of stillbirth among non-majority women has been reported even in countries where there is generally good access to medical care [100]. In Norway one group found women of non-Western origin to have increased risk for stillbirth, compared to women of Western origin (Western Europe except Turkey, North-America, Australia and Oceania) with an OR 2.2 (95% CI 1.3-3.8) [101]. The non-Western women more often neglected antenatal care and disregarded the advice of health-care workers, and inadequate communication was reported in 47% of the cases. They were also at greater risk of suboptimal care during delivery. Another author reported similar findings in USA with reduced prenatal-care utilization among black women [95]. Late attendance for antenatal care has been reported to explain some of the disparity in stillbirth rates in high-income countries [102].

### **Smoking**

There is a well documented association between smoking and fetal death, with increasing risk with increased amount of smoking and placental pathology as a proposed pathway [24,78,80,103]. The OR for the association of smoking and stillbirth is reported to be in the range 1.7-3.0 [23]. Smoking is a well known risk factor for placental abruption [104,105] and the higher rates of stillbirths among smokers appear mainly to be due to placental abruption and placenta previa [106]. There has also been reported a dose-related association between smokeless tobacco use and stillbirth [107], and studies from India have found an association between the use of biomass fuels for cooking and stillbirth [108].

A biological hypothesis regarding a causal relationship between smoking and stillbirth

has been proposed. Smoking increases the concentration of fetal carboxy-hemoglobin in addition to increasing the vascular resistance by the vasoconstrictive effect of nicotine and the reduced prostacyclin synthesis [109-111] probably contributing to placental pathology among smokers.

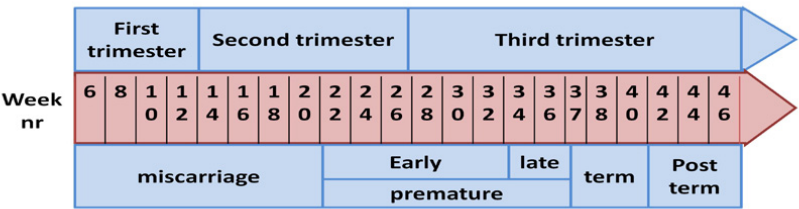
**Alcohol and recreational drugs**

Alcohol consumption has been associated with stillbirth [112], although this is not a consistent finding [80]. Cocaine use has been shown, in a meta-analysis, to increase the risk for stillbirth six-fold, probably due to an association with intrauterine growth restriction (IUGR) and placental abruption [113].

**2.1.5.2 Clinical risk factors**

**Gestational age**

**Figure 2.2.** Gestational age, terminology.



Live births peak at term whereas IUFDs occur with decreasing frequency from the 20th gestational week. The majority of stillbirths occur in the preterm period, that is, before 37 gestational weeks. Copper et al. reported in their material (USA) 51% of stillbirths occurring before week 28, 18% at term (37-41), and only 1% at 42 weeks or later [90]. Yudkin et al.

studied unexplained stillbirths after 28 gestational weeks and reported that over half of all stillbirths occurred before 37 weeks' gestation [57]. They reported the highest stillbirth *rates* (stillbirths per 1000 births) in the very preterm period (< 34 gestational weeks), the lowest at 39-40 gestational weeks' gestation, rising at 41 weeks and later. But paradoxically, although the stillbirth rates were highest early, the *risk* of stillbirth increased with advanced gestational age, peaking at 41 to 42 weeks. They observed this using a more appropriate method of calculating stillbirth risk related to gestational age. Rates are generally accepted when measuring risks. Stillbirth rates represent the proportion of stillbirths of total births. However, the population at risk for IUFD is not the population of delivered infants, but the population of unborn infants. Stillbirth risk is therefore better measured by the number of stillbirths divided by the number of unborn fetuses [57]. This is calculated by dividing the number of stillbirths at a given gestational week with the number of ongoing pregnancies at the same week [114]. By this measure reports show that even though the minority of stillbirths occur post-term (>42 weeks) the risk is relatively high because few pregnancies are still ongoing at such late gestational age [57,114]. Yudkin et al. showed in this way that the risk of stillbirth increased with maturity and was, for unexplained stillbirth after 28 gestational weeks, three times greater at 40 weeks than at earlier gestational ages [57]. Another study found the relative risk for antepartum stillbirth at 41 weeks to be 1.7 (95% CI 1.4-2.1) compared with the risk at 39 weeks [114]. The stillbirth risk in week 41 and 42 has been reported to be two- to ten-fold the risk in week 39 and 40 [57,114,115], but the results regarding the association of gestational age and stillbirth have not always been consistent. Huang et al. found, for unexplained stillbirth of fetuses weighing more than 500g, a gradually increasing risk of stillbirth from week 35 onward, but the risk at or after 41 weeks, was not significantly increased compared with the risk at 39-40 weeks [55]. Another study showed



similar findings, with a trend of progressively rising stillbirth risk with advanced gestational age, but the significance was not calculated [78].

### **Multiple pregnancies**

Multiple pregnancies pose an increased risk of all common pregnancy related complications, especially IUGR and preterm deliveries, in addition to complications specific to multiple pregnancies as twin to twin transfusion syndrome [116]. The risk of IUFD in twin pregnancies has been found to be four-fold the risk in singleton pregnancies [117], with reported risk estimates in the range 3.2-6.2 [49,75,76]. Multiple pregnancies constitute about 3% of births, but about 10% of stillbirths [36]. Higher order multiples have an even greater risk of perinatal death [118]. Lately multiple pregnancies have become more prevalent because of assisted reproduction techniques and higher maternal age, and will possibly gain more importance as a risk factor for stillbirth [119].

### **Premature labor**

Risk factors associated with preterm delivery are strong predictors of stillbirth since most stillbirths occur in the preterm period [90]. The most common causes of stillbirths between 24 and 27 weeks of gestation, reported from a Canadian material, were infection, placental abruption and lethal anomalies [47].

### **Placenta mediated pregnancy complications (PMPC)**

Stillbirth, placental abruption, IUGR, and hypertensive disorders are denoted as the “placenta mediated pregnancy complications”. They are thought to share the same pathogenesis of placental origin in many instances and tend to be associated with one another and share the same risk factors. The non-pregnant uterus has a high resistance circulation, but during the first half of a normal pregnancy, trophoblasts invade the spiral arteries changing the uterine circulation

dramatically, reducing the resistance [120]. Placental insufficiency probably originates/develops in very early pregnancy [121] and studies have suggested that complications in late pregnancy may be determined by impaired placental function already in the first 10 weeks after conception [122]. The flow in the uterine arteries can be assessed by Doppler flow velocimetry and a high resistance pattern of flow at the end of the second trimester is associated with an increased risk of IUGR and IUFD [123].

### *Placental abruption*

Placental abruption is defined as a premature separation of a normally implanted placenta and complicates about 1% of all pregnancies, but accounts for 10 – 20% of all fetal deaths [34,44]. It has been strongly associated with stillbirth in several studies with ORs in the range 11.4-18.9 [124,125].

The consequences of placental abruption vary from minor bleeding with little or no consequences, to a massive abruption leading to fetal death and severe maternal morbidity. Abruption involving more than 50% of the placenta is frequently associated with fetal death [126]. It appears that, in the vast majority of cases, abruption is the end result of a chronic process. Smoking, hypertension and SGA are strongly associated with placental abruption which indicates that problems with placentation are a common denominator for these conditions [126].

### *Intrauterine growth restriction (IUGR) / Small for gestational age (SGA)*

IUGR is used to describe a pattern of intrauterine fetal growth that deviates from expected norms, whereas SGA is based on birth weight and is often defined as birth weight below the 10<sup>th</sup> percentile for the gestational age [127]. To diagnose IUGR two ultrasound examinations, during the pregnancy, at least ten days apart are mandatory [127]. The two terms are not synonymous, as some SGA-fetuses may be constitutionally small while some normal-sized fetuses might be originally large fetuses that are IUGR. The use of population based reference curves of

birthweights underestimates IUGR among preterm births, and fails to distinguish between constitutional and pathological smallness [128]. The accurate detection of IUGR is improved with customized growth charts, which take into account maternal height and weight, parity, ethnic origin and the baby's gender [129].

IUGR is the condition most often associated with stillbirth (43%) and is found in the majority of stillbirths previously considered unexplained [46,50]. Poor fetal growth, without other environmental causes is assumed to indicate insufficient placental function. In a Canadian study the incidence of stillbirth among SGA fetuses was 46.8 per 1000, while the normal sized fetus had an incidence of 4.0 per 1000 (OR 11.8; 95% CI 8.1-17.1) [47]. Frøen et al. found that IUGR existed among 52% of all unexplained stillbirths compared to 13% of singleton liveborn controls (OR 7.0; 95% CI 3.3-15.1) [48], and they also found the distribution of risk factors in the group with IUGR to be different from the group without IUGR. Among women with IUGR-pregnancies smoking  $\geq 10$  cigarettes, maternal overweight (BMI  $>25\text{kg/m}^2$ ) and low education were associated with unexplained stillbirth, while in pregnancies without IUGR, smoking was not a risk factor, but maternal age  $\geq 35$  years, overweight and low education were. There appears to be a dose-response effect, with a greater risk of stillbirth with more profound SGA [128].

Women with a previous SGA pregnancy are at increased risk of stillbirth in the succeeding pregnancy, particularly if the infant was delivered preterm. A Swedish study found rates of stillbirths ranging from 2,4 per 1000 births among women whose first child was born at term and not SGA to 19 per 1000 births among women whose first child was born very preterm (before 32 weeks) and was SGA [130]. They found that the rate of stillbirth in the next pregnancy was lower for those with a previous stillbirth (7,6 per 1000 births) than for those with a previous SGA infant born moderately or very preterm (9,5 and 19 per 1000 births respectively).

### *Hypertensive disorders*

Hypertensive disorders (HD), comprising essential hypertension (HT), pregnancy related HT and preeclampsia complicate 5-10% of all pregnancies [131]. HT has been reported to have either no [47,90] or a very modest association with stillbirth [75,76,90] and Warland et al. even reported a decreased risk of stillbirth among women with HT [132]. One author found chronic HT, but not gestational HT, to be associated with stillbirth (aOR 3.46; 95% CI 1.1-10.5) [133]. Historically HT has been responsible for a notable proportion of fetal deaths, but optimal management has considerably reduced the risk of perinatal death associated with HT [134].

Preeclampsia complicates approximately 3% of all pregnancies and can induce serious complications for both mother and fetus. The consequences for mother and child depend on gestational age at onset, severity, and timing of diagnosis. Early onset (before gestational week 32) occurs among approximately 10% of the cases and is usually a sign of a more severe disease, with greater risk of complications [131]. Preeclampsia is associated with both IUGR and placental abruption.

### **Infertility**

Women who choose to delay childbearing are also more likely to have a history of infertility and are therefore more likely to conceive with reproductive technologies. There has been demonstrated an association between advanced reproductive technologies and perinatal mortality [23,118]. The more frequent multiple gestations in these pregnancies account for a significant portion of the increased risk. However, an increased risk has also been demonstrated in singleton pregnancies after in vitro fertilization or ovarian stimulation, associated with prematurity and low birth weight [135,136].

### **A history of stillbirth**

Women with a previous stillbirth have as much as 2-10 fold increased risk of a repeated stillbirth

compared to women with no previous stillbirths [34,91,137-139]. Women with a history of stillbirth have an increased risk of gestational diabetes, preeclampsia and IUGR, but these factors alone do not entirely explain the increased risk of a second stillbirth, although the risk depends on the etiology and gestational age of the prior stillbirth, presence of IUGR, and race [130,137]. Women with a history of other complications in previous pregnancies also have an increased risk of future stillbirth [130] and women with a previous stillbirth have been found to have an increased risk of other pregnancy complications in the subsequent pregnancy [140,141]. Heinonen et al. found that women with a history of stillbirth of other causes than maternal conditions or fetal abnormalities had more often placental abruption, preterm deliveries, and low-birthweight in the subsequent pregnancy [140]. Women with a previous fetal loss in gestational weeks 13 – 24 have also been found to have an increased risk of stillbirth in the succeeding pregnancy [142].

### **Previous Caesarean delivery**

A previous Caesarean section has been associated with an increased risk for stillbirth in a Scottish study (1992-1998) that demonstrated a 2-fold risk of antepartum stillbirth at 39 weeks' gestation compared with a previous vaginal birth [143]. The difference was mostly due to an excess of unexplained stillbirths. The results were confirmed among women delivering in the years 1999-2001, utilizing data from the same source [144]. An analysis of birth certificate data from USA was not able to demonstrate a similar association, but found quite the opposite [145], but the large study population was of an extremely heterogeneous nature. A meta-analysis of 6 studies demonstrated a 20% increase in the odds of stillbirth associated with previous Caesarean section [34], although confounding due to the indications of the Caesarean sections could not be excluded.

## **Maternal medical diseases**

Maternal medical disease can induce an increased risk of stillbirth, although better treatment in the last decades has reduced the risk associated with several conditions. Estimates suggest that maternal disease can contribute in 10% of fetal deaths [146]. Hypertension and diabetes mellitus (DM) are the most common medical problems complicating pregnancy and have earlier been associated with a great proportion of IUFD. However, good antenatal management reduces the risk of perinatal death associated with these conditions to a level only slightly above that of the general population [134]. Fretts et al. found that although the rates of stillbirth associated with insulin-dependent DM decreased from the 1960s to the 1980s, women with DM were still at a significantly increased risk for stillbirth in the 1980s [47]. Maternal hypertension, on the other hand, resulted in an increased risk of stillbirth in the 1960s, but not the 1980s [47]. Fretts reported, in a review article in 2005, ORs for the association with stillbirth to be 1.5-2.7 for chronic hypertension, 1.2-2.2 for DM treated with diet and 1.7-7.0 for insulin-dependent DM [23].

Other maternal medical diseases reported to be associated with stillbirths are: systemic lupus erythematosus (OR 6-20), renal disease (OR 2.2-30), thyroid disorders (OR 2.2-3.0), thrombophilia (OR 2.8-5.0), asthma, cardiovascular disease, and cholestasis of pregnancy (OR 1.8-4.4) [23,146]. Pregnancy loss associated with maternal medical disease typically occurs in women with severe disease.

### *Diabetes mellitus*

The majority of stillbirths associated with diabetes are reported to occur in the third trimester in patients with poor glycemic control and complications of macrosomia, polyhydramnion, IUGR and preeclampsia [147]. It appears that DM types 1 or 2 are more often associated with stillbirth than gestational diabetes [81,148]. In a review article from 2007, Silver summarized the risk of

stillbirth associated with diabetes and found OR 2.5 for DM type 1 and 2 but no association between gestational diabetes and fetal death [146]. However, some studies have not demonstrated an association between diabetes and stillbirth at all [75,76,90] and reports from referral centers suggest that with optimal management the risk of perinatal death associated with diabetes is only marginally above that of the general population [75]. Diabetic women have increased rates of fetal anomalies, hypertension and obesity, but the increased risk of fetal death persists after controlling for these factors [146]. One study demonstrated that women with a previous stillbirth more often had abnormal glucose tolerance and gestational diabetes in a subsequent pregnancy and speculated that undiagnosed gestational diabetes could possibly contribute to some unexplained stillbirths [141].

#### *Maternal hemoglobin concentration*

Plasma volume expansion and reduced hemoglobin concentration in the course of the pregnancy are normal physiologic responses to pregnancy. Both high and low hemoglobin concentrations have been associated with increased risk of perinatal death [149] and stillbirth [150,151]. Stephansson et al. found both low ( $\leq 11.5$  g/dL) and high ( $\geq 14.6$  g/dL) hemoglobin concentrations in early pregnancy to be associated with an increased risk of stillbirth and a relatively large decrease in hemoglobin concentration during pregnancy seemed to be protective [151]. Elevated hemoglobin in early pregnancy and failure of significant hemodilution resulted in a 2-fold increase in stillbirth risk. High hemoglobin level ( $\geq 14.6$  g/dL) at first antenatal visit was specially associated with SGA stillbirths or preterm stillbirths [151]. Frøen et al. demonstrated that women with hemoconcentration, with the lowest hemoglobin measured during the pregnancy greater than 13.0 g/dL, had a 9-fold increase in the risk of unexplained fetal death [150].

## **2.2 Thrombophilia**

### *Definition*

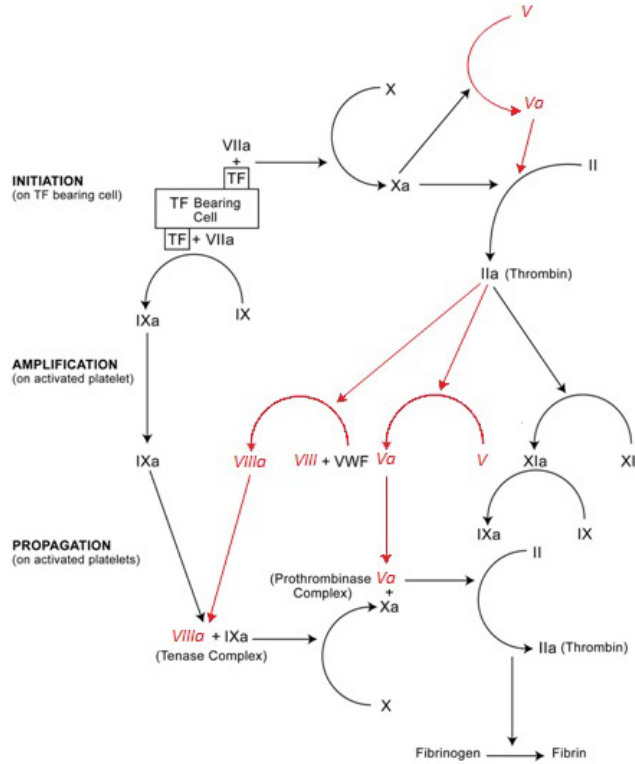
Thrombophilia is a hereditary or acquired abnormality of blood coagulation that predisposes an individual to developing thrombosis, either venous or arterial. The hemostatic abnormalities do not necessarily cause thrombosis, but may weaken the ability to cope with a hypercoagulable insult. The risk of thrombotic complications is determined by many other factors in addition to thrombophilia, and the presence of several risk factors simultaneously is likely to augment the thrombotic risk [152,153].

### **2.2.1 Coagulation**

The coagulation cascade describes a series of reactions that ultimately lead to the generation of thrombin, which in turn cleaves fibrinogen. The three phases of the coagulation (cell-based model) are: the initiation-, amplification- and propagation phases (Figure 2.3). Cell-based coagulation is initiated when flowing blood, with factor (F) VII, comes in contact with cells expressing tissue factor (TF). TF is expressed by fibroblasts and smooth muscle cells surrounding the endothelium and is normally not exposed to circulating blood [154]. An injury of a vessel leads to the formation of a platelet clot and the exposure of TF to circulating blood leading to activation of the coagulation. Once bound to TF, FVII is activated (FVIIa). The activated TF/FVIIa complex activates FX and FIX. The coagulation process eventually leads to the conversion of prothrombin to thrombin [154]. FXa remaining on the cell surface, with FVa as a cofactor, converts prothrombin to thrombin. At first small amounts of thrombin are produced, which plays a key role in the amplification phase when thrombin activates platelets in addition to FV, FVIII, and FXI which in turn results in greater amounts of thrombin being produced [154,155]. This occurs on the surface of the activated platelets.



**Figure 2.3.** Cell-based model of coagulation. With permission from Vine AK, 2009 [154].



Of most importance in the propagation phase are the tenase- and prothrombinase-complexes, which both are assembled on the surface of activated platelets and are dependent on negatively charged phospholipids and calcium [155]. Activated FVIII (FVIIIa) is the co-factor of FIXa and together they form the tenase-complex. The tenase-complex activates FX which then forms the prothrombinase-complex with FVa as a cofactor [154]. The prothrombinase-complex cleaves prothrombin, thus producing great amounts of thrombin, which in turn converts soluble fibrinogen into fibrin to form a hemostatic fibrin clot, stopping the bleeding.

Simultaneously with the activation of the coagulation, anticoagulation systems are

activated to prevent uncontrolled coagulation and thrombosis. The three most important systems are the TF pathway inhibitor (TFPI) system, the antithrombin (AT) system, and the protein C (PC)/ protein S (PS) system. The TFPI-system inhibits the TF/FVIIa-complex, inhibiting the initiation phase [156]. AT inhibits all activated coagulation enzymes including thrombin, FIXa, FXa and FVIIa (when bound to TF). This inhibition is greatly accelerated by heparins. PC is activated by thrombin in combination with an endothelial cell receptor (EPCR, endothelial PC receptor) and thrombomodulin. As thrombin binds to thrombomodulin it loses the ability to convert fibrinogen to fibrin. Activated PC (APC) in combination with PS inactivates FVa and FVIIIa [154,155].

### **2.2.2 Inherited thrombophilia**

#### *Definition*

Hereditary thrombophilia is an inherited abnormality, based on a genetic mutation in the genes coding for either coagulation factors such as FV and prothrombin, or anticoagulants such as AT, PC or PS. The genetic abnormalities result either in “gain of function” (coagulation factors) or “loss of function” (coagulation inhibitors).

#### *Factor V Leiden (F5 rs6025)*

The Leiden variant of coagulation FV (FV<sub>Leiden</sub>) is caused by a polymorphism in the gene coding for FV, a substitution of adenine for guanine at nucleotide 1691 in exon 10 of the F5 gene, which predicts an arginin by glutamine replacement at position 506 in the mature FV molecule. This polymorphism causes a change in the structure of FV that has important consequences for the function of FV both in the coagulation and anticoagulation pathways. The APC-mediated inactivation of FVa is impaired, in addition to impairment of the cofactor function of FV in the APC-mediated inactivation of FVIIIa [157,158]. This results in a slower degradation of FVa and

FVIIIa and an increased risk of thrombosis. This condition is defined as APC-resistance. 95% of cases of APC-resistance are caused by FV<sub>Leiden</sub> [159]. Heterozygosity for the polymorphism is the most common heritable thrombophilia in Caucasian populations and is found in 2-15% of the general population, more prevalent in northern than in southern Europe [160,161]. In Norway the prevalence of factor V Leiden has been reported to be 7-8% [162,163]. FV<sub>Leiden</sub> is associated with relatively mild thrombophilia and individuals heterozygous for the polymorphism have a five to ten-fold increased risk for thrombosis compared to non-carriers [164]. Homozygosity results in a more severe thrombophilia with 50 to 100-fold increased risk of thrombosis [164].

#### *Prothrombin gene G20210A polymorphism (F2 rs179963)*

Prothrombin is a vitamin-K dependent protein which is converted by the prothrombinase complex to thrombin in the propagation phase of the coagulation [154]. The prothrombin gene G20210A polymorphism (prothrombin polymorphism) causes increased serum concentrations of prothrombin which leads to increased risk of thrombosis [165]. Approximately 1% of blood donors in Oslo, Norway have this polymorphism [162], but prevalences in the range 2-6.5% are reported in Europe, higher in southern than northern Europe [165,166]. Asymptomatic carriers of the polymorphism have a similar incidence of venous thrombosis as carriers of FV<sub>Leiden</sub>.

#### *Antithrombin deficiency*

AT deficiency is the most severe thrombophilic condition and causes a profound increase in thrombosis risk [167]. Several point mutations in the gene coding for AT cause either reduced concentrations of the protein (type I antithrombin deficiency) or reduced activity (type II) [168]. Heterozygous type I deficiency is associated with an approximately 10-fold increased risk of thrombosis. No case of homozygous type I deficiency has been described suggesting that complete AT deficiency is incompatible with life [169]. Heparin accelerates the inhibition rate of AT 1000-fold. A study among Canadian blood donors estimated a prevalence of 0.2% [170], but

prevalence of 0.02% has also been demonstrated [171]. AT levels are unchanged in normal pregnancy [172].

#### *Protein C deficiency*

PC is an anticoagulant protein, produced in the liver, vitamin-K dependent, activated by thrombin. In its activated form it mediates the inactivation of FVa and FVIIIa. Many different mutations can cause deficiency of the protein by either reduced activity or quantity. Deficiency results in reduced inactivation of FVa and FVIIIa and therefore an increased risk of thrombosis [173]. The prevalence was found to be 0.2% in a study of Scottish blood donors [174]. Pregnancy does not modify PC levels [175].

#### *Protein S deficiency*

PS is an anticoagulant protein, mainly derived from liver synthesis. About 60% is bound to C4b binding protein, but only free PS has anticoagulant properties [176]. Deficiency can be caused by reduced total and free PS, reduced APC cofactor activity, or normal total PS but reduced free PS [173]. PS acts as a cofactor for APC and together they inactivate FVa and FVIIIa [154]. The prevalence of PS deficiency is demonstrated to be 0.03-0.13% [177]. Levels of the C4b-binding protein are increased in pregnancy, with the combined oral contraceptive pills and inflammation, leading to a reduction in the concentration of free PS. Reduced concentrations of PS associated with these conditions [178,179] and variations in PS levels related to age and gender can cause misdiagnosis [180,181]. However, recent reports have demonstrated that women using second generation oral contraceptives have almost normal PS levels and that lowering the reference values results in better specificity and minimizes misclassification [182].

#### *Other inherited thrombophilias*

A number of polymorphisms of other coagulation proteins have been associated with venous

thromboembolic disease, but these will not be discussed here.

### **2.2.3 Acquired thrombophilia**

#### *Definition*

Acquired thrombophilia refers to a group of conditions, not congenital, that may develop throughout life and augment the risk of thrombosis. These include, among other conditions: pregnancy, cancer, surgery, infection, hypovolemia, thrombocythemia, and antiphospholipid antibodies (APAs) [183].

#### *Antiphospholipid antibodies*

APAs, a heterogeneous group of acquired autoimmune antibodies, are associated with acquired thrombophilia, relevant in the case of pregnancy complications. Of particular interest are lupus anticoagulant (LA), anti- $\beta_2$ -glycoprotein 1 antibodies (anti- $\beta_2$ -GP1) and anti-cardiolipin antibodies (aCL). They recognize epitopes expressed by phospholipid-binding proteins or protein-phospholipid complexes. They can in vitro act as coagulation inhibitors in phospholipid dependent coagulation assays. The binding of antibody-antigen complexes to negatively charged phospholipids on the cell membrane, in vivo, induces a procoagulant state due to inhibition of anticoagulant reaction and cell-mediated events.

The APAs are relevant for the Antiphospholipid Syndrome (APS), the most common cause of acquired thrombophilia [167]; an antibody mediated thrombotic disorder, which is defined by venous or arterial thrombosis and/or specific pregnancy complications in patients with persistently positive tests for APAs [184]. The prevalence of APAs (LA or aCL) in the general population, is found to be in the range 1-5% among young healthy controls [185], but there are discrepancies between individual studies due to assay differences, definition of cut-off values and referral bias.

### **2.3 Thrombophilia and IUFD**

Successful outcome of pregnancy is dependent on the development of adequate placental circulation. This includes adequate trophoblast invasion into the uterine vasculature and the development and maintenance of an adequate utero-placental circulation. Abnormalities in placental vasculature as inadequate invasion by the trophoblast or damage to the maternal vessels supplying the placenta lead to impaired flow and prothrombotic changes which may result in pregnancy complications, such as miscarriages, preeclampsia, IUGR, placental abruption or IUFD denoted as the “placenta mediated pregnancy complications” (PMPC) [186,187].

Major physiological changes of hemostasis occur during normal pregnancy; increased concentrations of the procoagulant factors FVIII, FV and fibrinogen, reduced anticoagulant activity with reduced concentrations of PS and acquired APC-resistance in addition to reduced fibrinolytic activity [187,188]. These adaptations are necessary for the development of an adequate placental circulation and to secure adequate hemostasis during delivery and the post-partum period. When pregnancy has been established, the normal placental circulation is maintained by a dynamic balance between the coagulation and fibrinolytic systems. The backside of these adaptations is that they also induce an increased risk of thrombosis, greater among women with thrombophilia.

Fetal loss in women with thrombophilia could be explained by excessive thrombosis of the placental vessels, placental infarction and secondary uteroplacental insufficiency [189]. However, it is unlikely that hypercoagulability with thrombosis of placental vasculature is the main pathophysiological pathway. Other mechanisms are probably involved, since adverse pregnancy outcome can occur in thrombophilic women without placental thrombosis [190]. Prevention of implantation by inadequate trophoblast invasiveness or damage to decidual or chorionic vessels is described for the APS [191]. Suggested mechanisms are by alteration of cell

surface adhesion molecules of the maternal vessel wall or trophoblasts and damage to trophoblasts by activation of the complement system or a pro-inflammatory response [192].

Kupferminc et al. reported in 1999 that 65% of women with some form of PMPC had positive tests for heritable or acquired thrombophilia [193]. In pregnancies with signs of placental insufficiency, like IUGR or placental infarction, thrombophilia is more likely to contribute to a fetal death [194] and the co-existence of thrombophilias appears to increase the risk of obstetric complications [195]. However, thrombophilia is common in normal individuals. Therefore one should be cautious in attributing stillbirth to thrombophilia in women who test positive as most of them will never experience a stillbirth.

### **2.3.1 Inherited thrombophilia and IUFD**

Inherited thrombophilias have been associated with an increased risk of stillbirth in the third trimester, although causality has not yet been reasonably documented [196,197]. The proposed mechanisms are by 1) microthrombosis in the placental bed, causing placental infarctions and a subsequent compromise of the feto-maternal circulation or 2) impeding the initial placentation process by increased generation of thrombin and consequently excess generation of fibrin and fibrin degradation products that can induce apoptosis of trophoblasts resulting in early fetal loss or later placental insufficiency and IUFD [189,198-200]. Case series and retrospective studies have reported increased risk of IUFD associated with the FV<sub>Leiden</sub> polymorphism [8,193], G20210A polymorphism of the prothrombin gene [201,202] and deficiencies of the anticoagulant proteins; AT, PC and PS [8,203]. However, these findings are not consistent and no large population based studies have been performed to evaluate these associations. The inconsistencies between studies may reflect small sample size, inconsistent case definition and differences in the definition of IUFD/stillbirth. The results of individual studies have been

summarized in meta-analyses. Robertson et al. [6] demonstrated an association between fetal loss in the third trimester and heterozygous FV<sub>Leiden</sub> (OR 2.06; 95% CI 1.1-3.86, based on 6 studies), heterozygous prothrombin polymorphism (OR 2.66; 95% CI 1.28-5.53, based on 5 studies), and PS deficiency (OR 20.09; 95% CI 3.7-109.15, based on 2 studies) [6]. The risk associated with AT deficiency and PC deficiency was not found statistically significant, based on 1 and 2 studies respectively [6]. A meta-analysis of Rey et al. from 2003 showed similar results and included many of the same studies [5]. They found an association of FV<sub>Leiden</sub> (OR 3.26; 95% CI 1.82-5.83) and non-recurrent fetal loss after 19 weeks of gestation, the prothrombin polymorphism (OR 2.3; 95% CI 1.09- 4.87) and PS deficiency (OR 7.39; 95% CI 1.28-42.83) and non-recurrent fetal loss after 20 and 22 weeks of gestation respectively. Two of six studies for FV<sub>Leiden</sub>, two of five for prothrombin polymorphism and two of three for PS deficiency showed significant associations with IUFD.

#### *Factor V Leiden*

Of interest was that none of the individual studies included in the meta-analysis of Robertson et al. [6], addressing the association of FV<sub>Leiden</sub> and late fetal loss, found significant associations. Martinelli et al. studied 67 women with unexplained fetal death at or after 20 gestational weeks [201], Meinardi et al. registered fetal loss after 20 gestational weeks in a cohort of 228 carriers of FV<sub>Leiden</sub> [204] and Rothbart et al. studied 14 women with IUFD after 24 weeks without apparent explanation. The study of Bare et al. registered intrauterine death among 128 FV<sub>Leiden</sub> carriers [205], Agorastos et al. studied eight women with stillbirth after 24 gestational weeks [206], and Many et al. investigated 40 women with IUFD at or after 27 weeks [202]. A population based study in Germany also failed to demonstrate an association between FV<sub>Leiden</sub> and stillbirth in the third trimester [207], as did a prospective study of Clark et al. [208], but Clark's study only included 22 stillbirths at or after 24 weeks of gestation. Two studies included in the meta-



analysis of Rey et al. found a significant association of FV<sub>Leiden</sub> and IUFD [5]. The study of Gris et al. of 232 women with one or more unexplained fetal loss after 22 gestational weeks demonstrated an OR 4.8 (95% CI 1.8-12.4) for the association and Kupferminc et al. who studied 12 women with stillbirth after 23 gestational weeks found an OR 4.9 (95% CI 1.1-22.3) [8,193].

#### *Prothrombin gene G20210A polymorphism*

The association of the prothrombin polymorphism and stillbirth appears to be more consistent than the association with FV<sub>Leiden</sub>. Nevertheless, of five individual studies included in the meta-analysis of Robertson et al. the three following studies did not show significant associations: Gris et al. studying 232 women with one or more unexplained fetal loss after 22 gestational weeks [8], Kupferminc et al studying 16 women with stillbirth after 23 gestational weeks [209], and Alonso et al. investigating 8 women with IUFD at or after 23 gestational weeks [210]. The two studies that found significant associations were the study of Martinelli et al. who studied 67 women with fetal death at or after 20 weeks of gestation with OR 3.16 (95% CI 1.02-9.75) [201] for the association and the study of Many et al. who found an OR of 2.3 (95% CI 1.3-4.0) among 40 women with unexplained IUFD at or after 27 weeks of gestation [202].

#### *Antithrombin, Protein C and protein S deficiencies*

Few studies have been able to study the association of AT, PC or PS deficiencies and IUFD, since the prevalence of these deficiencies is low and the sample size of individual studies is usually small. Preston et al. (the EPCOT-study) found that six of 108 women with AT deficiency compared to six of 395 women with no known inherited thrombophilias had stillbirths in the third trimester (> 28 weeks), OR 5.2 (95% CI 1.5-18.1) [211], but they did not find stillbirth to be significantly more prevalent among women with PC deficiency. The meta-analysis of Rey et al. was not able to analyze the association of AT or PC deficiencies and fetal loss at different gestational ages, but no significant associations were found with fetal loss in general [5].

Many et al. found an association with PS in a study of 40 women with unexplained IUFD at or after 27 gestational weeks. Three of 40 cases and none of the 80 controls had PS deficiency (OR 3.2; 95% CI 2.4-4.1) [202]. Gris et al. did also find a positive association among 232 women with one or more unexplained stillbirths after 22 gestational weeks with an OR 23.05 (95% CI 2.96-179.62) [8].

### **2.3.2 Antiphospholipid antibodies and IUFD**

Specific pregnancy outcomes and complications are features of the antiphospholipid syndrome, namely: a) one or more unexplained deaths of a morphologically normal fetus at or beyond 10<sup>th</sup> week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or b) one or more premature birth of a morphologically normal neonate before the 34<sup>th</sup> week of gestation because of: (i) eclampsia or severe preeclampsia defined according to standard definitions, or (ii) recognized features of placental insufficiency, or c) three or more unexplained consecutive spontaneous abortions before the 10<sup>th</sup> week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded [184].

Retrospective and prospective studies have found APAs to be associated with fetal loss and it is documented that 15% of women with recurrent pregnancy loss have APAs [8,212,213]. An association between a circulating anticoagulant and pregnancy loss was first reported in the 1950s [214]. In 1985 the presence of aCL was first associated with pregnancy loss and Lockshin suggested subsequently that aCL was a more sensitive test than LA to identify women at risk of pregnancy loss [215]. In 1989 a large study reported an association of LA with recurrent spontaneous abortion and fetal death after 10 gestational weeks [216]. The lack of concordance between assays for LA and aCL has indicated the importance of obtaining results from both

assays [217]. APAs have also been demonstrated, in prospective studies, to be associated with other pregnancy complications [218,219].

It appears that the association of APAs with early fetal loss is better documented than the association with stillbirth. Results of individual studies on the association of APAs and stillbirth have been inconsistent, but the sample size is often limited. One of the first reports focusing on late fetal loss (at or after 20 gestational weeks) was published in 1994. Bocciolone et al. studied 99 women with unexplained fetal death at or after 20 gestational weeks and found a significant association between aCL (OR 21.0; 95% CI 1.21-364.5), but not LA (OR 8.06; 95% CI 0.43-151.85) [6,7]. Comparison of individual studies has been difficult because of methodological differences between studies, like different selection of cases and controls, different assays, and different definitions of reference values. The results of studies have never the less been attempted summarized in meta-analyses. A meta-analysis of Robertson et al. from 2005 found an association between aCL, but not LA, and stillbirth in the third trimester, OR 3.3 (95% CI 1.62-6.7), based on 6 studies and 2.38 (95% CI 0.81-6.98), based on 3 studies, respectively [6]. Three of the six individual studies, including the study of Bocciolone et al. (mentioned earlier), reporting on aCL found a significant association with late fetal loss [7,8,220]. De Carolis et al. studied 75 women with IUFD after 20 weeks and Gris et al. investigated 232 women with one or more unexplained IUFDs after 22 gestational weeks. Among the three studies that did not find a significant association Yasuda et al. analysed aCL in the first trimester among 860 pregnant women and prospectively registered fetal deaths after 24 weeks and other pregnancy complications. Two aCL positive and one aCL negative women experienced stillbirth [221]. In the study of Alfrevic et al. one of 18 women with unexplained stillbirth after 23 gestational weeks and three of 44 controls were aCL-positive and Infante-Rivard et al. investigated 23

women with fetal loss after 28 weeks, of which none were positive for aCL [221-223]. Only the study of Gris et al. showed a significant association between LA and stillbirth [8].

## **2.4 Prevention of stillbirths in high-income countries**

The interventions to prevent stillbirths fall in three main strategic areas: 1) improving the health and well-being of women, 2) detection and management of women at risk during pregnancy and 3) improvement of information and standards of maternity care [27]. The cornerstone of prevention of intrapartum stillbirths is the management of labor and delivery. Intrapartum stillbirths have almost disappeared since intrapartum fetal monitoring was introduced. For the prevention of antepartum stillbirths optimal timing of delivery is crucial, but in order to accomplish that, pregnancies at risk/risk factors have to be detected. This may be achieved with high quality antenatal care, accessible to all women [27]. Either elimination of the risk factor, if possible, and/or surveillance in continued pregnancy with optimal timing of delivery will hopefully prevent an IUFD. However, the most common error in reducing potentially preventable stillbirths has been demonstrated to be failure to adequately diagnose and manage IUGR and to recognize maternal medical risk factors [224].

Tests of fetal well-being can potentially assist in decision making regarding timing of delivery. Various methods of fetal antepartum monitoring exist; fetal movement counting, non-stress test (cardiotocogram (CTG)), stress test (contraction based CTG), biophysical profile, ultrasound and Doppler flow velocimetry (measures blood flow dynamics in uterine, umbilical and fetal arteries). In at-risk pregnancies assessment of fetal growth by ultrasound should be considered since antepartum surveillance with optimal timing of delivery has been demonstrated to improve outcomes in IUGR pregnancies [225]. The use of Doppler flow velocimetry of umbilical and fetal arteries in high risk pregnancies has also been demonstrated, in a meta-

analysis, to be associated with a 29% overall reduction in perinatal mortality, although a specific effect on stillbirths was not significant [226]. However, no methods of fetal monitoring have been shown to reduce the risk of stillbirth when used for screening in unselected populations [38] and data regarding the cost-effectiveness of stillbirth prevention are limited [23].

A screening test that detects women at increased risk for stillbirth also demands an effective intervention among those high-risk women. If the intervention is ineffective there is not much point in screening for the condition. Therefore, when results of tests of fetal well-being are assessed, possible interventions, often induction of labor or Caesarean section have to be included in the analysis. Neonatal mortality rates have to be included as well since they most probably will be affected by the interventions, especially scheduled deliveries in the premature period. A model showing that losses might be prevented by structured fetal assessment and routine induction of labor among women at increased risk for stillbirth has been described by Fretts et al. [73]. However, available tests of fetal well-being do not have the predicted values assumed in this model [38]. Controversial is that the last decades early stillbirths (<28 gestational weeks) have been the most difficult to prevent, but most forms of antenatal testing are recommended implemented from gestational weeks 32-34.

The oldest method to assess fetal well-being, and the most commonly used, is maternal perception of fetal movements [227]. However, the efficacy of organized fetal movement counting, with pre-defined alarm limits, to prevent stillbirths in the general population is controversial. Moore et al. reported a considerable reduction in stillbirth rates during a 7 month study period of prospective evaluation of a fetal movement screening program and concluded that the count-to-ten fetal movement screening was simple and effective in reducing the stillbirth rate [228]. Results of randomized controlled trials in mixed-risk populations have been inconsistent, but the authors seem to agree that fetal movements probably reflect fetal well-being

and that awareness among pregnant women regarding fetal movements is wise [229,230]. Studies on the efficacy of fetal movement counting are difficult to conduct, since study participation automatically raises awareness regarding fetal movements as was demonstrated in one study that showed decreased total stillbirth rate in both study groups in the study period [229]. However, Frøen et al. has demonstrated an increased risk of IUGR, fetal distress in labor, and stillbirth in low-risk pregnancies with decreased fetal movements [231]. Further research is needed regarding the role of maternal assessment of fetal movements, especially in high-risk pregnancies, but increased awareness by recommending women to count fetal movements on a daily basis has been associated with significant benefits [227].

Several medical treatments for certain causes of antepartum stillbirth have been assessed, of which none is in routine practice [38]. Low molecular weight heparin might be effective in the case of thrombophilia but no high quality data exist on the effects, although studies are being conducted. Low molecular weight heparin and low dose acetylsalicylic acid are currently only recommended for the prevention of recurrent pregnancy loss in women with APAs [195,232-234].

### **3. Aims of the study**

The aims of the current thesis were:

- To estimate the incidence of IUFD in a Norwegian population.
- To assess socio-demographic and clinical risk factors for IUFD, by the use of two different control groups with different selection of variables.
- To evaluate changes in risk estimates by different control selection.
- To classify stillbirths in a Norwegian study-population according to the CODAC classification of perinatal deaths, and to estimate risk factors according to cause of death.
- To investigate the association between APAs and a history of IUFD.
- To investigate the association of inherited thrombophilia and IUFD.





## **4. Material and methods**

This thesis is based on an epidemiologic study of women with IUFD. It was conducted in two parts. The first part (papers I and IV) was an epidemiologic retrospective case-control study focusing on incidence and classification of stillbirths and sociodemographic and clinical risk factors. Study subjects in this part of the study were women with IUFD and two different control groups comprising women with live births, one including all women with live births in the same area and time period as the cases and the other comprising conditionally selected controls from one hospital. The second part of the study (papers II, III and IV) was a case-control study investigating the association of acquired or inherited thrombophilia and IUFD. The study subjects were women with a history of IUFD and controls with live births only, who agreed to participate and donated blood samples and answered questionnaires.

The present study was a part of a larger hospital-based case-control study: the Venous Thromboembolism In Pregnancy (VIP) study and was registered as a clinical observational study at [www.clinicaltrials.gov](http://www.clinicaltrials.gov), registration number NCT00856076. The other part of the study investigated the epidemiology and risk factors for pregnancy associated venous thrombosis. Data on clinical and biochemical risk factors for venous thrombosis related to pregnancy have been published previously [163,235-238].

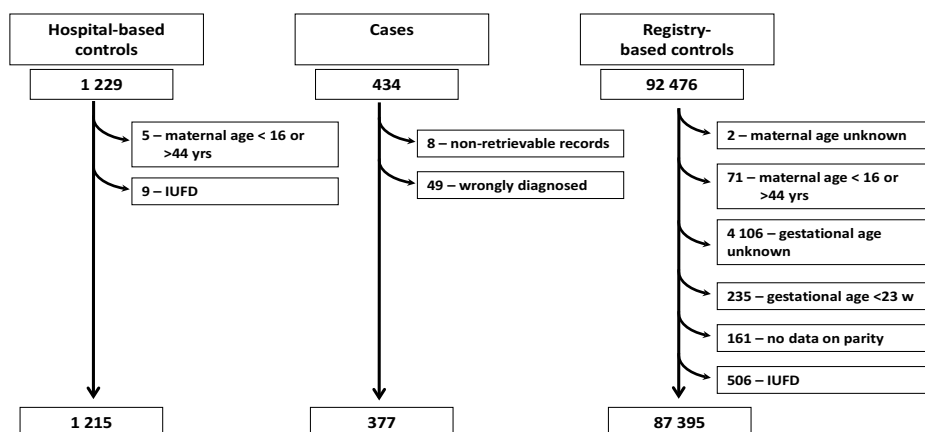
### **4.1 Identification of cases**

#### **4.1.1 First part of the study (papers I and IV)**

All women with a diagnosis of IUFD, from January 1<sup>st</sup> 1990 through December 31<sup>st</sup> 2003, at the Departments of Obstetrics at the Oslo University Hospital Ullevål (OUH), Oslo, and Akershus University Hospital (AHUS), Nordbyhagen, Norway, were identified retrospectively by search for selected codes of the WHO's ICD versions 9 or 10, that were registered in the patient

administrative systems at the respective hospitals. The codes searched for were; ICD-9: 656.4 IUFD and ICD-10: O36.4 IUFD. We identified 434 possible cases of IUFD defined as fetal death in singleton or duplex pregnancies at  $\geq 23$  completed gestational weeks or birthweight  $\geq 500$ g irrespective of gestational age. The medical records were retrieved and reviewed for validation of the diagnosis of IUFD. The records were non-retrievable for eight cases and 49 were wrongly diagnosed, leaving us with 377 women with a verified diagnosis of IUFD (Figure 4.1).

**Figure 4.1.** Flowchart of the selection of cases and controls in the first part of the study.

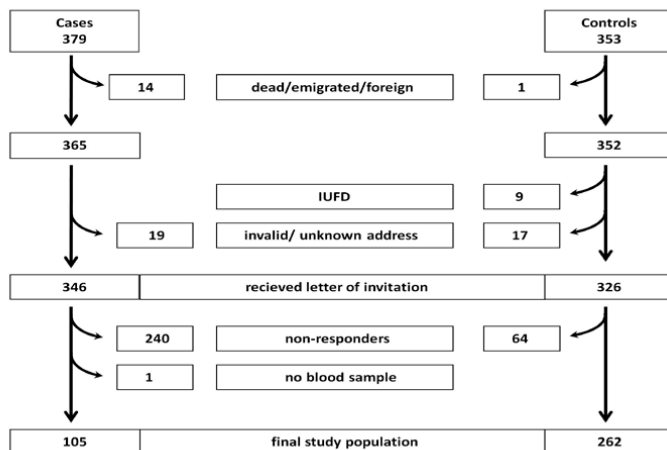


#### 4.1.2 Second part of the study (papers II, III and IV)

The 377 women with a verified diagnosis of IUFD in singleton or duplex pregnancies from the first part of the study were eligible for participation in the second part. Additionally two women with IUFD at the study hospitals in the study period, but not identified earlier, who contacted the study after having heard of it through the Norwegian SIDS and Stillbirth Society were eligible for participation. The women's unique personal identification numbers (given to each Norwegian

citizen at birth or on immigration) were merged with census data (Statistics Norway, Oslo, Norway). Women who had emigrated, died, or had an invalid, unknown or foreign address, were excluded from the study, leaving us with 346 cases eligible for study participation (Figure 4.2).

**Figure 4.2.** Flowchart of the selection of cases and controls in the second part of the study.



The eligible participants were approached during 2008 by a letter outlining the purpose of the study. Those interested contacted us by e-mail or telephone to schedule an appointment to donate a blood sample and to answer a questionnaire. One woman did not donate a blood sample and was therefore excluded from the study. After two reminders the final case population comprised 105 women (Figure 4.2).

## 4.2 Selection of controls

### 4.2.1 First part of the study (paper I and IV)

Two control groups with different selection criteria and with different sources of information on

potential risk factors were chosen for comparison.

#### ***4.2.1.1 Facility-based controls***

The Norwegian Medical Birth Register (MBR) comprises data from compulsory notification of all births after 16 weeks of gestation, registered at each hospital and forwarded to the MBR. Information from the MBR revealed 92 476 singleton or duplex deliveries in the study period at the two study-hospitals. Excluded from analysis were women with no data for maternal age (n=2), women younger than 16 years or older than 44 years since none of the cases were in those age groups (n=71), women who had missing data on gestational age (n=4 106), women at gestational age less than 23 weeks in combination with fetal weight < 500g (n=235), women with no data on parity (n=161), and women with IUFD in the index pregnancy (n=506). The remaining 87 395 women served as the facility-based controls (Figure 4.1).

#### ***4.2.1.2 Selected controls***

In all 1 229 women with live singleton or duplex births at OUH in the study period, were selected as controls for both arms of the VIP-study [235]. For each woman with venous thrombosis in the pregnancy or the post-partum period within the study period four women, giving birth at the same time, were selected from the MBR as possible controls. The two women first listed served as controls, but if one or both of their medical records were not retrievable, the third and/or fourth women were chosen as control(s). We excluded three women older than 44 years, two younger than 16 years and nine women with IUFD in the index pregnancy, leaving us with 1 215 women as selected controls (Figure 4.1).

#### **4.2.2 Second part of the study (papers II, III and IV)**

The 1 229 women selected as controls for the VIP-study were initially invited to participate in the venous thrombosis part of the study. The eligible participants were during 2006 approached

by a letter outlining the purpose of the study. Those interested contacted the study by e-mail or telephone to schedule an appointment to donate a blood sample and to answer a questionnaire. A total of 353 (28.7%) women agreed to participate and donated blood samples and answered questionnaires. At the same time they agreed on receiving an invitation and a new questionnaire at a later time for participation in the IUFD-part of the study. Of the 353 women nine women had a history of IUFD and 18 had emigrated, died, or had an invalid, unknown or foreign address and were excluded from the study, leaving 326 women eligible for participation. They received an invitation together with a new questionnaire by postal mail. After two reminders 262 women had returned the questionnaire and thus comprised the control population (Figure 4.2).

### **4.3 Collection of data**

#### **4.3.1 Sociodemographic and clinical variables**

The cases and the selected controls were identified at the participating hospitals. The medical records were retrieved and reviewed for validation of the diagnosis of IUFD and information on demographics, general health, obstetric history, index pregnancy, delivery, post-mortem examination of the fetus, histological examination of the placenta, and laboratory data. Individual data were transferred to a case-report-form (CRF). The CRFs were scanned, consistency analysis run and invalid data entries corrected after a review of relevant medical records. The data for the facility-based control group were provided by the MBR.

Risk factors assessed were delivery hospital, period of delivery, maternal age, parity, marital status, assisted reproductive therapy, smoking habits, previous medical disorders (type 1 diabetes mellitus, essential hypertension and thyroid disease), twin pregnancy, gestational diabetes, gestational hypertensive disorders, placental abruption, placenta previa, premature rupture of membranes, SGA and gestational age at delivery.

The period of delivery was divided in two groups (1990-1999 and 2000-2003). Maternal age was analyzed either in four (< 29, 30-34, 35-39, or  $\geq 40$ ) or two (< 35 or  $\geq 35$ ) groups. Parity was also analyzed in either four (0, 1, 2, or  $> 2$ ) or two (0 or  $\geq 1$ ) categories. Marital status was analyzed in two categories (single or married and cohabiting) and smoking was defined according to smoking status at first antenatal visit (smoker or non-smoker). The assisted reproductive therapy variable included in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (yes or no). The expected date of delivery was estimated by routine ultrasound examination at 16 to 18 gestational weeks for 73% of the women. In the absence of ultrasound data, gestational age was determined by the first day of the last menstrual period. The time of fetal death was determined by the gestational age at diagnosis. A diabetes variable was analyzed in three categories (pre-gestational diabetes (DM), gestational diabetes (GD) or no diabetes), but in Paper IV it was analysed in two categories (diabetes or no diabetes). The hypertensive disorder (HD) variable was also analysed in three categories (all hypertension (HT) (essential or gestational), preeclampsia (PE) (all forms) or no HD). Validated population-based growth charts were used to determine whether the fetus was SGA, which was defined as birthweight below the 2.5<sup>th</sup> percentile for gestational age at birth. Other variables were dichotomized in categories with or without the relevant condition. Individuals with missing data were allocated to the reference group for the respective variables. Data on smoking, SGA and thyroid disease were not available for the facility-based controls. In model C (see chapter 4.4.1, page 67) of the multivariate analyses an interaction was revealed between SGA and HD and therefore a new variable combining the two conditions was created, comprising four categories: 1) no HT, no PE, no SGA (reference group), 2) HT or PE, no SGA, 3) HT or PE with SGA and 4) SGA, no HD.

#### **4.3.2 Classification of stillbirths**

The primary cause of fetal death (COD), and associated conditions (AC) when appropriate were assigned by the author according to the CODAC classification for perinatal deaths (described in more detail in chapter 2.1.4.1, page 22-25) [60] after reviewing all available data on each case. Two of the main groups were not relevant for coding in this study (group 9=termination and group 1=neonatal death).

#### **4.3.3 Placenta histology**

The results of the original placenta examinations, available in the medical journal for 93% (351/377) of the cases, were reviewed. This revealed that the placentas had originally been evaluated by general pathologists. No standardized protocols for macro- or microscopic evaluation or sampling of placental tissue were in use in the study period and the reports were inconsistent. In most cases sections had been sampled from the umbilical cord, the membranes and minimum two sections from placental tissue from areas with and without focal parenchymal pathology. The tissue sections had routinely been fixed in formalin, processed and embedded in paraffin blocks, and 3.5 µm sections stained with Hematoxylin-Eosin (HE). For more uniform placenta investigations, to obtain a more accurate classification of causality, the original placenta specimens were retrieved and reassessed, if available. 268 specimens were retrievable and were reassessed by two experienced pathologists (Borghild Roald and Gitta Turowski) with special interest in placental pathology at the Department of Pathology, OUH Ullevål. They were blinded regarding the identification and clinical details of the stillbirths. Specimens with microscopic signs of acute or chronic villitis and/or intervillitis were immunostained with a standard panel of antibodies to T-cell- and histiocytic markers. If the specimens were not available for reassessment the original placenta histological descriptions (n=99) were used. A histological

description of the placenta, either the reassessment or the original one, was available for 367/377 (97.3%) of the cases. Placenta information was most often missing from the controls since histological examination of the placenta is rarely carried out among normal birth giving women.

#### **4.3.4 Blood sampling and analysis**

The cases donated blood samples in 2008 and the controls in 2006. Blood was collected in 5 mL Vacutainer tubes (Becton-Dickinson, Meylan-Cedex, France) containing 0.5 mL buffered citrate (0.129 mol/L) and 5 mL Monovette tubes containing ethylenediaminetetraacetic acid (EDTA) (Sarstedt, Nümbrecht, Germany). The Monovette EDTA tubes were frozen immediately and the tubes with buffered citrate were centrifuged at 2000g for 15 minutes within 1 hour, and plasma aliquots frozen and kept at -70 °C until assayed.

The blood was analysed for LA, aCL, anti- $\beta_2$ GP1, FV<sub>Leiden</sub>, prothrombin polymorphism, AT, PC and PS. The assays were performed at the Hematologic Research Laboratory, Department of Hematology, OUH Ullevål. LA was identified using validated in-house lupus ratio (LR) tests, which are automated, quantitative, integrated tests for LA [239,240]. Two LR tests were performed, one based on the activated partial thromboplastin time (LR-APTT) and the other on the Russell's viper venom time (LR-RVVT). The tests were performed in a 1:1 mixture of patient plasma and pooled normal plasma. For each test two coagulation times were measured, one with a reagent with low and the other with a reagent with high phospholipid concentrations. The ratio between the two coagulation times (low phospholipid/high phospholipid concentration) was divided by the corresponding ratio obtained with pooled normal plasma. The final ratio is defined as the LR of that patient's plasma [240]. The reagents were made from different concentrations of natural phospholipids (crude cephalin, generously provided by Dr. Tore Janson, Axis-Shield PoC AS, Oslo, Norway). In the APTT-based assay, a constant concentration



of ellagic acid (Sigma-Aldrich, St. Louis, Missouri, USA) was used as an activator of coagulation. In the RVVT-based test, Russell's viper venom (RVV) (Sigma-Aldrich, St. Louis, Missouri, USA) activates factor X directly. The 99<sup>th</sup> percentile of the LR of the control group was chosen as the upper reference limit, and was 1.22 for the APTT-based LR test and 1.19 for the RVVT-based test. Women with LR above the upper reference limit for one or both of the LR-tests were considered LA positive.

aCL IgG and IgM isotypes were analyzed with in-house enzyme-linked immunosorbent assays (ELISA), essentially as described by Gharavi et al. [241]. We used serial dilutions of an in-house control drawn from a strongly aCL positive patient, which were standardized against Harris' commercial standards (American Diagnostica Inc., Stamford, CT, USA). Values for IgG and IgM isotypes of aCL were reported in GPL and MPL units, respectively. The cut-off values for a positive test were defined by the 99<sup>th</sup> percentile of the values of the control group, and were 10.7 for aCL IgG and 23.7 for aCL IgM. Women with values above the cut-off limits for aCL IgG and/or aCL IgM were considered as aCL positive.

Anti- $\beta_2$ GP1 IgG and IgM were assayed with commercial ELISA kits (QUANTA Lite<sup>TM</sup>  $\beta_2$  GP1 IgG/IgM, INOVA Diagnostics Inc., San Diego, USA) for semi-quantitative determination. Results were expressed in standard IgG and IgM anti- $\beta_2$ GP1 units, SGU and SMU, respectively. The 99<sup>th</sup> percentiles of the values of the control group were defined as upper reference limits, and were 6.5 for anti- $\beta_2$ GP1 IgG and 30.3 for anti- $\beta_2$ GP1 IgM. Women with values above the upper reference limits for IgG and/or IgM were considered anti- $\beta_2$ GP1 positive.

DNA was extracted using a DNA isolation kit I large volume (Roche Diagnostics, Basel, Switzerland) using Magna Pure (Roche Diagnostics). FV<sub>Leiden</sub> and the prothrombin polymorphism were detected with FV<sub>Leiden</sub> Mutation Detection and Prothrombin Mutation Detection kits (Roche Diagnostics), respectively, run on a real-time polymerase chain reaction

(PCR) - analyser (LightCycler 2.0, Roche Diagnostics).

AT and PC activities and free PS quantities (antigen assay) were analyzed with commercial kits, Coamatic® Antithrombin, Coamatic® Protein C and HemosIL™ Free Protein S (Chromogenix, Lexington, MA, USA). AT activity < 80% and PC activity < 70% were defined as deficient. PS values were expressed as percentage of normality. The reference limit for PS was defined as two standard deviations below the mean of the control group or 53.7%. Women with values below the reference limit were defined as PS deficient. One woman with PS and one with both PC and PS below cut-off limits reported use of warfarin medication and were therefore excluded from all analyses including inherited thrombophilia (n = 103).

#### **4.3.5 Questionnaire**

In the second part of the study (papers II and III) the participants answered questionnaires regarding socio-demographic factors, obstetrical history, general and psychological health, and quality of life at the present time. The cases answered the questionnaire on site at the time of the blood sampling, but the controls received and returned the questionnaire by postal mail. The questionnaires were scanned, consistency analysis run and invalid data entries corrected after a review of the relevant questionnaires.

#### **4.4 Statistical analysis**

Incidence was estimated as the number of IUFDs per thousand deliveries with 95% confidence interval (CI). Prevalences were reported in percentages. Risk factors were analysed by comparing prevalences of potential risk factors between cases and controls with chi-squared tests, Fisher's exact tests and univariate and multiple logistic regression. Results of risk factor analyses were presented as crude odds ratios (OR) and adjusted OR (aOR) with 95% CIs.

Interactions between significant factors were tested at 95% significance level ( $p < 0.05$ ). Significance level was set at  $p < 0.05$ . All data was analyzed using the Statistical Package for Social Science version 16.0 or 18.0 (SPSS Inc, Chicago, IL, USA).

#### **4.4.1 Paper I**

We assessed risk factors for IUFD by comparing cases and controls in three multiple logistic regression models (table 5.1, page 70). Model A compared the cases with facility-based controls. Variables included in the model were chosen based on the significance of each variable in the univariate analysis and variables moderately associated with the outcome ( $p\text{-value} < 0.15$ ) were considered for inclusion into the model. The significance of each variable and effects on the stability of the model, were investigated by backward and forward stepwise logistic regression. Model B explored the same variables as included in Model A, but using the selected controls in the comparison, whereas Model C compared the cases with selected controls including additional variables. The additional variables in Model C were variables, for which there were no data among the facility-based controls, only among the selected controls. The additional variables were added to model C based on the same principles as described for Model A.

#### **Gestational age**

The probability of IUFD according to gestational age was defined by the number of events (number of IUFDs) divided by the number of pregnancies at risk at a defined gestational age. The population at risk in a given gestational week consisted of all ongoing pregnancies. The estimates of probability for IUFD according to gestational age were derived from the following: the number of ongoing pregnancies at the beginning of gestational week  $n$  ( $P_n$ ), the number of all births in gestational week  $n$  ( $B_n$ ), and the number of IUFDs in gestational week  $n$  ( $S_n$ ). The conditional probability of IUFD at gestational week  $n$  ( $PS_n$ ) was estimated by the number of

IUFDs in that week, divided by the number of ongoing pregnancies at the beginning of the week minus half the births in the given week as in this equation [114]:

$$PS_n = S_n / (P_n - (0,5 \times B_n)).$$

Relative risk of IUFD in a given gestational week was derived from the conditional probability of IUFD in that week divided by the conditional probability of IUFD in gestational week 40.

#### **4.4.2 Paper IV**

For detection of potential variations in risk factors according to cause, risk factors were analysed by comparing prevalences in different causal groups with prevalences in the control group. The eight actual COD groups were combined and analysed in three groups: placental causes (N=190), unknown causes (N=73), other causes (the remaining six causal groups combined in one) (N=114). ORs for each risk factor were adjusted by all the other relevant risk factors in a multiple logistic regression model. Variables included in the regression models were chosen based on the same principles as described in chapter 4.4.1. The Results were presented as percentages, ORs and aORs with 95% CIs.

#### **4.5 Ethical aspects**

The Regional Committee for Medical Research Ethics, Region East, Norway, approved the study. Authorization for the use of information from medical records for research purposes was obtained from the Norwegian Ministry of Health and Social Affairs. The Norwegian Data Inspectorate approved the use of data comprising sensitive personal health information, and the merging of clinical and register data by using the unique 11-digit personal identification number. Women participating in the study by answering questionnaires and donating blood samples signed a written informed consent.

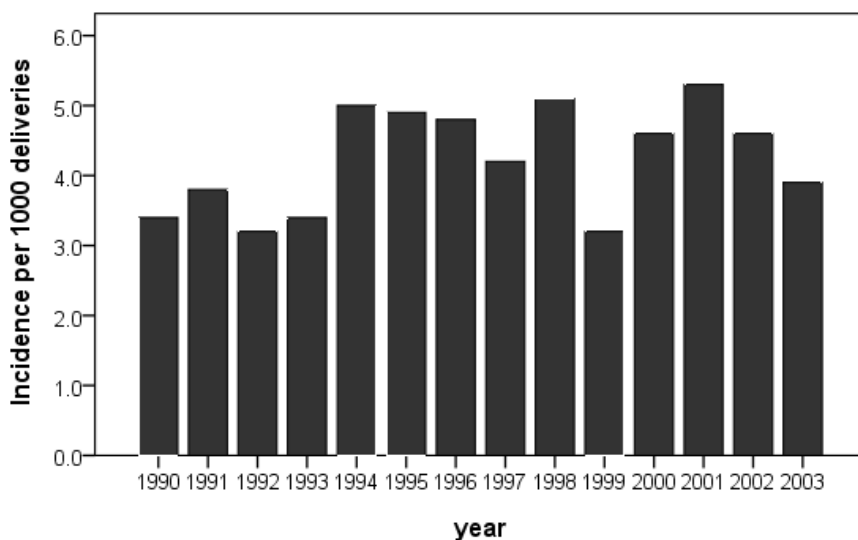
## 5. Summary of results

### 5.1 Incidence and risk factors of fetal death in Norway: a case-control study, Paper I

In Paper I we studied the incidence of IUFD and socio-demographic and clinical risk factors, among the cases and two differently selected control groups.

The incidence of IUFD after 22 gestational weeks was 4.1 per 1000 deliveries (95% CI 3.7-4.5) and did not change significantly over the study period (Figure 5.1). For comparison, the incidence at or after 28 gestational weeks in our material was 3.3 per 1000 (95% CI 2.9-3.6).

**Figure 5.1.** Yearly incidence of IUFD at OUH Ullevål and AHUS 1990-2003.



There was a significant difference in the incidence of IUFD between the two hospitals; 4.7 (95% CI 4.1-5.3) per 1000 births at OUS and 3.4 per 1000 (95% CI 2.8-4.0) at AHUS. When comparing with facility-based controls in a multivariate analysis (Table 5.1, Model A) maternal age over 39, single civil status, twin pregnancy, hypertensive disorders, pre-pregnancy and gestational diabetes mellitus, placental abruption and placenta previa were significant risk factors

**Table .5.1** Risk factors for IUFD by different control groups and variable selection. Adjusted odds ratios (aOR) with 95% confidence intervals (CI).

	Model A	Model B	Model C
	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
<b>Cases = N</b>	377	377	377
<b>Controls = N</b>	87 395	1 215	1 215
<b>Time period</b> 1990 – 1999 2000 – 2003	n.s.		n.s.
<b>Age (years)</b> < 29 30-34 35-39 ≥ 40	0.9 (0.7-1.1) =Reference 1.3 (0.9-1.7) <b>2.5 (1.6-4.0)</b>	1.0 (0.8-1.3) =Reference 1.0 (0.7-1.4) 0.9 (0.6-1.7)	0.9 (0.7-1.3) =Reference 1.1 (0.7-1.6) 0.7 (0.3-1.4)
<b>Parity</b> 0 1 2 > 2	n.s.		n.s.
<b>Twin pregnancy</b>	<b>1.6 (1.0-2.4)</b>	<b>3.3 (1.8-6.0)</b>	<b>2.9 (1.4-6.0)</b>
<b>Civil status</b> Not married/cohabiting	<b>1.9 (1.4-2.6)</b>	<b>1.9 (1.3-2.8)</b>	1.3 (0.8-2.1)
<b>Hypertensive disorders (HD)</b> Preeclampsia (PE) Hypertension (HT)	<b>2.9 (2.2-3.9)</b> <b>1.9 (1.2-2.8)</b> <b>5.6 (3.8-8.7)</b>	<b>1.7 (1.2-2.4)</b> 1.3 (0.8-2.2) <b>2.1 (1.3-3.3)</b>	
<b>HD/ SGA</b> No HD, no SGA HD, no SGA HD with SGA SGA, no HD			=Reference <b>1.6 (1.0-2.6)</b> <b>11.5 (5.2-25.3)</b> <b>51.5 (27.4-96.7)</b>
<b>Diabetes</b> Pre-pregnancy diabetes Gestational diabetes	<b>4.0 (1.9-8.8)</b> <b>2.5 (1.1-5.7)</b>	<b>4.8 (1.5-15.4)</b> 1.8 (0.7-4.9)	<b>5.0 (1.4-17.5)</b> <b>3.0 (1.1-8.4)</b>
<b>Placental abruption</b>	<b>22.0 (15.8-30.8)</b>	<b>16.0 (7.9-32.5)</b>	<b>15.4 (7.3-32.3)</b>
<b>Placenta previa</b>	<b>3.4 (1.3-8.8)</b>	<b>2.8 (0.9-8.7)</b>	<b>3.4 (1.0-11.9)</b>
<b>Smoking</b>			<b>2.6 (1.9-3.7)</b>
<b>Thyroid disease</b>			<b>4.6 (2.0-10.6)</b>

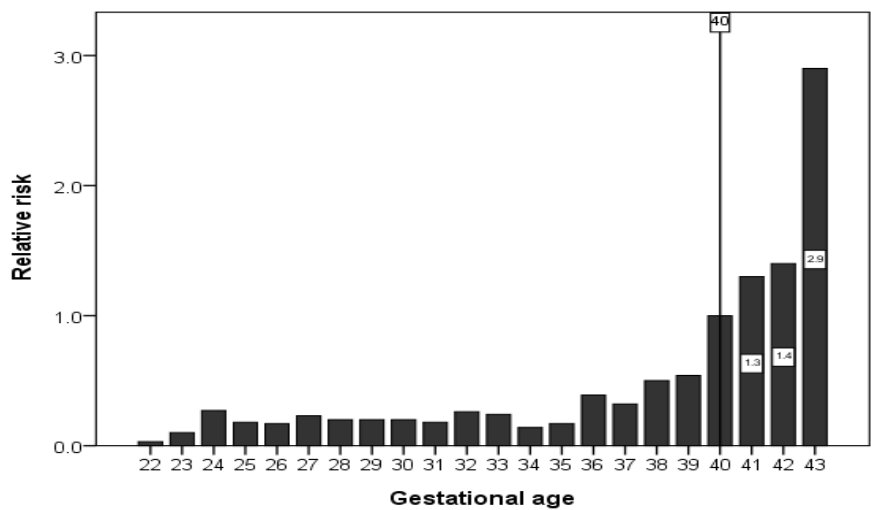
Model A: Cases compared with facility-based controls adjusted for hospital, age, multiple pregnancy, civil status, hypertensive disorders, diabetes, placental abruption, and placenta previa. Model B: Cases compared to selected controls, same variables as in model A. Model C: Cases compared to selected controls with additional variables, adjusted for age, multiple pregnancy, civil status, hypertensive disorders/SGA, diabetes mellitus, placental abruption, placenta previa, smoking, and thyroid disease.

n.s.: not significant.

for IUFD. When comparing with the selected controls, some additional variables were available for analyses (Table 5.1, Model C). The following factors were significant in the final model: twin pregnancy, hypertensive disorders, SGA, pre-pregnancy and gestational diabetes mellitus, placental abruption, placenta previa, smoking and thyroid disease. SGA and placental abruption were the strongest risk factors for IUFD. Hypertensive disorders were of low risk if not associated with SGA. Other risk factors were of low to moderate risk. Advanced maternal age was a significant risk factor when compared with facility-based controls, but not when comparing with the selected controls and the risk estimates for hypertension were higher when comparing with the facility based controls. Overall, the risk estimates pointed in the same direction independent of control-group.

**Gestational age**

**Figure 5.2.** Relative risk of IUFD with the conditional risk at 40 gestational weeks as a reference.



The relative risk (RR) of IUFD in each gestational week, compared with the risk at 40 gestational weeks, is displayed in Figure 5.2. The RR increased gradually from week 36-37, with

RR 1.3 (95% CI 0.8-2.2), 1.4 (95% CI 0.6-3.2) and 2.9 (95% CI 0.7-12.1) in weeks 41, 42 and > 42 respectively. We did not find advanced gestational age ( $\geq 42$  gestational weeks) to be a significant risk factor for IUFD.

## 5.2 The association of antiphospholipid antibodies with intrauterine fetal death: a case-control study, Paper II

In Paper II we studied the prevalences of APAs and their association with a history of IUFD. The APAs studied were: LA, aCL (IgG and IgM), and anti- $\beta_2$ GP1 (IgG and IgM). The study population consisted of 105 women with a history of IUFD and 262 controls with live births only.

**Table 5.2.** Prevalence of APAs (%) and ORs with 95% CIs for the association of APAs with IUFD.

Variable	Cases N= 105 % (n)	Controls N =262 % (n)	OR	95% CI
APA positive – $\geq 1$ tests	9.5 (10)	5.0 (13)	2.0	0.9-4.8
LA positive – $\geq 1$ test	4.8 (5)	1.1 (3)	4.3	1.0-18.4
Anti- $\beta_2$ GP1 positive	4.8 (5)	1.5 (4)	3.2	0.8-12.3
aCL positive	3.8 (4)	2.3 (6)	1.7	0.5-6.1

Among women with a history of IUFD 9.5% were positive for at least one APA test compared to 5.0% of the controls (Table 5.2). Having a single positive APA test was not significantly associated with IUFD (OR 1.5; 95% CI 0.6-4.0), whereas positivity for a combination of more than one APA-tests produced higher odds (OR 7.9; 95% CI 0.8-76.5), but nevertheless not a significant association. Women with a history of IUFD were significantly more often positive for LA compared to controls with live births only (OR 4.3; 95% CI 1.0-



18.4). Dividing the LA positive women into those positive for LA only and those positive for aCL and/or anti- $\beta_2$ GP1 in addition to LA revealed that the risk related to LA seemed to be confined to women positive for LA in combination with other APA. Being positive for anti- $\beta_2$ -GP1 or aCL alone was not significantly associated with a history of IUFD after 22 weeks.

### 5.3 The association of inherited thrombophilia and intrauterine fetal death: a case-control study, Paper III

In Paper III we studied the association of inherited thrombophilia and IUFD. The following thrombophilias were investigated: FV<sub>Leiden</sub>, prothrombin polymorphism, AT-, PC- and PS-deficiencies. The study population consisted of 103 women with a history of IUFD and 262

**Table 5.3.** Prevalences of inherited thrombophilia (%) and ORs with 95% CIs for the association of thrombophilia with intrauterine fetal death, compared to controls.

Variable	Cases N= 103 % (n)	Controls N =262 % (n)	OR	95% CI
Factor V Leiden	10.7 (11)	7.6 (20)	1.4	0.7-3.1
Prothrombin polymorphism	5.8 (6)	1.5 (4)	<b>4.0</b>	<b>1.1-14.4</b>
Antithrombin deficiency	1.0 (1)	0 (0)	-	-
Protein C deficiency	1.0 (1)	1.1 (3)	0.8	0.09-8.2
Protein S deficiency	1.9 (3)	2.3 (6)	0.8	0.17-4.3
Any inherited thrombophilia	18.4 (19)	11.8 (31)	1.7	0.9-3.1
$\geq 2$ inherited thrombophilias	1.9 (2)	0.8 (2)	2.6	0.4-18.5

controls with live births only. 18.4% of the cases and 11.8% of the controls were positive for at least one inherited thrombophilia (Table 5.3). Women with a history of stillbirth after 22 gestational weeks were more often carriers of the G20210A prothrombin polymorphism

compared to women with live births only (OR 4.0; 95% CI 1.1-14.4). FV<sub>Leiden</sub>, AT-, PC- or PS-deficiencies were not significantly associated with IUFD after 22 weeks of gestation. Two cases (1.9%) and two controls (0.8%) had a combination of two inherited thrombophilias.

#### **5.4 Classification of stillbirths by cause of death and risk factor analysis - an observational case-control study, Paper IV**

In Paper IV we classified the stillbirths by cause according to the CODAC classification of perinatal deaths and studied socio-demographic, clinical and thrombophilic risk factors in different causal groups. The study populations comprised 377 cases and 1 215 controls or, in the case of thrombophilic risk factors, 105 cases and 262 controls.

A main COD (Level I) according to CODAC was assigned to all the cases (N=377) and was placental in 50.4% of the cases, unknown among 19.4% and infectious in 12.2%. Other causes were: intrapartum (0.5%), congenital anomalies (6.1%), fetal (2.1%), cord (8.9%) and maternal (1.3%). The largest subgroups (level II of the main COD) within placental causes were; abruption or retroplacental hematoma (32.1% of placental causes, 16.2% of all stillbirths) and infarctions and thrombi (35.3% of placental causes and 17.8% of all stillbirths). These two groups were responsible for 34% of all IUFD after 22 gestational weeks.

An AC was assigned to 72.4% (273/377) of the cases. The most frequent ACs were; placental in 92/377 (24.4%), cord in 51/377 (13.5%) and associated perinatal conditions in 55/377 (14.6%). A placental AC was assigned to 68 (18% of all cases) with a non-placental COD. Thus 258/377 (68%) of all cases had either a placental COD or placental ACs. Among the cases with an unknown cause of IUFD, 35.6% (26/73) had a placental associated condition.

To detect socio-demographic and clinical risk factors 377 cases classified in different causal groups were compared with 1 215 controls. Smoking and SGA were significant

**Table 5.4.** Risk factors analyses, cases according to main COD compared to controls. Adjusted odds ratios (aOR) with 95% confidence intervals (CI).

<b>Variable \ COD</b>	<b>Placenta N=190</b>	<b>Unknown N=73</b>	<b>Other N=114</b>	<b>All cases N=377</b>
	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
<b>Civil status</b> Not married/cohabiting			<b>2.1 (1.1-3.9)</b>	
<b>Twin pregnancy</b>	<b>3.6 (1.3-9.4)</b>	<b>5.0 (1.5-16.0)</b>	2.2 (0.6-8.0)	<b>3.0 (1.4-6.3)</b>
<b>Hypertensive disorders</b> Preeclampsia/eclampsia Hypertension	0.5 (0.2-1.1) <b>2.2 (1.0-4.8)</b>	0.2 (0.06-1.0) <b>3.5 (1.5-8.0)</b>	0.2 (0.07-0.8) 1.0 (0.3-2.9)	0.6 (0.3-1.2) <b>1.9 (1.1-3.4)</b>
<b>Diabetes</b>	<b>3.5 (1.1-11.3)</b>	<b>8.7 (3.0-25.4)</b>	2.9 (0.8-10.8)	<b>3.8 (1.7-8.4)</b>
<b>Placental abruption</b>	<b>42.0 (19.4-91.0)</b>	-	1.4 (0.2-11.5)	<b>16.1 (7.6-33.9)</b>
<b>Smoking (at first visit)</b>	<b>2.5 (1.6-3.9)</b>	<b>3.7 (2.1-6.6)</b>	<b>2.5 (1.5-4.1)</b>	<b>2.6 (1.9-3.8)</b>
<b>Small-for-gestational age</b>	<b>47.9 (26.6-86.1)</b>	<b>28.6 (12.8-63.6)</b>	<b>38.6 (19.9-74.8)</b>	<b>32.9 (20-54.2)</b>
<b>Thyroid disease</b>	<b>5.5 (2.0-15.6)</b>	3.8 (0.8-18.0)	<b>5.5 (1.7-17.6)</b>	<b>4.8 (2.1-11.0)</b>

Each variable adjusted for the all the other variables in each logistic regression model.

risk factors in all causal groups. Twin pregnancy, hypertension and diabetes were detected as significant risk factors among women with IUFD of placental or unknown causes. Placental abruption, not surprisingly, was a significant a risk factor in the placental group only and single civil status only among women with IUFD of other causes. Thyroid disease was associated with an increased risk of stillbirths of placental and of other causes (Table 5.4).

The 105 cases and 262 controls who donated blood samples for the biomarker study were equally distributed in the main COD groups as the whole study-population (47.6% with placental and 20% with unknown causes).

Inherited thrombophilia, a composite result, was significantly associated with IUFD of placental causes, OR 2.2 (95% CI 1.0-4.7) and the prothrombin polymorphism was more

**Table 5.5.** Prevalences of acquired and inherited thrombophilia by different causes of stillbirth.

Variable \ COD	All N=105 *N = 103	Placenta N = 50 *N=49	Unknown N = 21 *N=21	Other N = 34 *N=33	Controls N =262
	% (n)	% (n)	% (n)	% (n)	% (n)
<b>*Factor V Leiden</b>	10.7 (11)	14.3 (7)	14.3 (3)	3.0 (1)	7.6 (20)
<b>*Prothrombin polymorphism</b>	5.8 (6)	8.2 (4)	0	6.1 (2)	1.5 (4)
<b>*Antithrombin deficiency</b>	1.0 (1)	0	0	3.0 (1)	0
<b>*Protein C deficiency</b>	1.0 (1)	0	0	3.0 (1)	1.1 (3)
<b>*Protein S deficiency</b>	1.9 (2)	2.0 (1)	0	3.0 (1)	2.3 (6)
<b>*Any inherited thrombophilia</b>	18.4 (19)	22.4 (11)	14.3 (3)	15.2 (5)	11.8 (31)
<b>LA</b>	4.8 (5)	4.0 (2)	4.8 (1)	5.9 (2)	1.1 (3)
<b>aCL</b>	3.8 (4)	2.0 (1)	4.8 (1)	5.9 (2)	2.3 (6)
<b>Anti- <math>\beta_2</math> GPI</b>	4.8 (5)	6.0 (3)	0	5.9 (2)	1.5 (4)
<b>Any APA</b>	9.5 (10)	6.0 (3)	9.5 (2)	14.7 (5)	5.0 (13)
<b>*Any thrombophilia</b>	27.2 (28)	28.6 (14)	23.8 (5)	27.3 (9)	16.4 (43)

\*Two women reported use of Warfarin and were therefore excluded from all analyzes of inherited thrombophilias.

prevalent among women with placental COD and other causes (Table 5.5), but statistically significant only in the group of placental causes, OR 5.7 (95% CI 1.4-23.8). The prevalence of FV<sub>Leiden</sub> was double among women with placental or unknown COD compared to the controls, although this did not reach statistical significance. Being positive for APAs was significantly associated with a history of IUFD of other causes (OR 3.3; 95% CI 1.1-9.9). Similar proportions, 4.0 – 5.9%, of women in all causal groups were LA-positive, compared to 1.1% of the controls, but the association did not reach significance in any separate causal group although the ORs in the different groups were in the range 3.6 – 5.4.

## **6. Methodological considerations**

### **6.1 Paper I and IV**

#### **6.1.1 Identification of cases**

The cases were identified by ICD codes in the patient administrative systems at the participating hospitals. It is possible that some cases were not detected by this method as inaccurate coding is probably unavoidable. However, diagnoses registered in the patient administrative systems are easily verified by accessing the medical records and falsely diagnosed IUFDs were thus excluded. Diagnoses of other conditions in the medical history or related to the pregnancy could be verified as well. Another possibility would have been identifying the cases in the MBR, but IUFD in the MBR is defined as fetal loss after 16 gestational weeks and underreporting of stillbirths is a known problem [personal communication: J.F.Frøen, M.D, Ph.D., Norwegian Institute of Public Health]. Information in large databases like the MBR is also reported to be less accurate than data from medical records [242,243].

We chose to define stillbirth in our study as 23 or more completed gestational weeks or a fetus of at least 500 g irrespective of the gestational age. The WHO definition of stillbirth is a fetus  $\geq 500$  g, or 22 or more completed weeks of gestation [13]. Birth weight takes priority over gestational age since birth weight is thought to be more reliably reported. However, in many instances, especially in high-income countries, the use of the gestational age is preferred. This leads to higher reported stillbirth rates as many fetuses weigh less than 500 g at 22 weeks [16].

#### **6.1.2 Selection of controls**

##### ***Selected controls***

The selected controls were selected for both arms of the VIP-study (thrombosis and IUFD). They

were women, without a known venous thrombosis, delivering at the same time as each case of a woman with a pregnancy related venous thrombosis. The only exclusion criterion at the time of selection was a history of venous thrombosis. We find it unlikely that this selection was a source of bias in the first part of our study, when investigating socio-demographic and clinical risk factors. However, it could have been a source of bias in the second part of the study, when examining the association of thrombophilia and IUFD.

This control group was for practical reasons selected from only one of the study hospitals. This might have caused a selection bias as the delivering populations at each of the hospitals were not necessarily alike. Our data on maternal age revealed in fact that there probably was a selection bias. When we compared the cases with the facility-based controls (all women delivering at the study hospitals in the study period), advanced maternal age was a significant risk factor for IUFD, not detected when comparing the cases with the selected controls. We looked further into this and compared the age distribution of the different control groups and found that the selected controls were on average older than the women in the facility-based control group. The selected controls did not accurately represent the age of the background population from which the cases were selected. However, this did probably not affect the risk estimates for the other variables as maternal age was adjusted for in the multivariable analyses.

### ***Facility-based controls***

The facility-based controls were not a selection of women, but represented the entire birth-giving population of the study hospitals, from which our cases were extracted and thus comprised the “true” demographic distribution. Using them as controls thus minimized or eliminated the risk of selection bias making the results more generalizable. But there can be a disadvantage using this control group regarding the quality of data extracted from the MBR, as will be discussed later.

## 6.2 Paper II, III and IV

### 6.2.1 Selection of cases

**Table 6.1.** Prevalences of social and clinical factors at index pregnancy among cases participating and those not.

Variable	Cases participating N= 105	Cases Non-participating N =274	p*
	%	%	
<b>Time period</b>			
1990-1999	62.9	57.7	
2000-2003	37.1	42.3	n.s.
<b>Age</b>			
< 35	80.0	76.6	
≥ 35	20.0	23.4	n.s.
<b>Parity</b>			
0	52.4	51.5	
1	34.3	30.7	
≥ 2	13.3	17.9	n.s.
<b>Civil status (at registration)</b>			
Married or cohabiting	90.5	85.0	n.s.
Not married/cohabiting	9.5	15.0	
<b>Multiple pregnancy</b>	3.8	6.6	n.s.
<b>Hypertensive disorders</b>			
Preeclampsia/ eclampsia	5.7	7.7	
Hypertension	4.8	9.9	n.s.
<b>Diabetes</b>			
Diabetes type 1 or 2	1.0	2.2	
Gestational diabetes	0.0	2.2	
All diabetes	1.0	4.4	n.s.
<b>Placental abruption</b>	8.6	12.8	n.s.
<b>Placenta previa</b>	1.9	1.1	n.s.
<b>Smoking (at first visit)</b>			
Non-smoker	71.4	61.7	
Smoker	28.6	38.3	n.s.
<b>Small for gestational age</b>	35.2	35.8	n.s.

Eligible for participation in the second part of the study were 346 women. Of the total 379 with IUFD in the study period 105 (28%) agreed to participate. This low response-rate increases the risk of selection-bias. We had the advantage of having information from medical records from all the eligible participants and were thus able to compare the women participating with those not participating. We found no significant differences in socio-demographic or clinical factors between the groups (Table 6.1). Therefore we do not believe that this low participation rate was a

basis for a serious selection bias, but it did affect the power of the study. Stillbirth is a traumatizing experience which women for various reasons may not wish to be reminded of many years after the incidence. Contributing to the low response-rate might also have been the inconvenience of having to present at the hospital in order to donate a blood sample and answer questionnaires. It is possible that women with negative long-term outcomes to a larger degree declined to participate in the study. On the other hand, women that had adequately coped with the loss and moved forward may also have found little interest in participating.

### **6.2.2 Selection of controls**

The 353 controls eligible for the second part of the study were already a selected group, as they had donated blood samples two years earlier and at the same time agreed to answer a new questionnaire related to this part of the study. The participation rate in this part of the study was therefore 74% (262/353), but if taken into account all the 1 215 women originally eligible as controls, the participation rate was 21.6%. As for the cases, we had information from medical records from all the 1 215 eligible participants. We compared the participating 262 controls with the 953 non-participating women. The participating controls were older, of lower parity, more often married or cohabiting, smoked less often and had more often preeclampsia (Table 6.2). Other factors were evenly distributed between the groups. We find it unlikely that the prevalence of thrombophilia would be affected by these parameters, apart from preeclampsia. Thrombophilia is possibly associated with preeclampsia. If so, this would result in a higher prevalence of thrombophilia among the participating controls and thus a possible underestimation/attenuation of the association between thrombophilia and stillbirth.

Women with venous thrombosis were excluded from the control group at the time of selection. Because of this, it is possible that thrombophilia was less prevalent in the control



group than expected in the general population. This could in return result in an overestimation of the association between thrombophilia and IUFD.

**Table 6.2.** Social and clinical factors at index pregnancy among controls participating and those not.

Variable	Controls participating N=262	Controls Non-participating N=953	p <sup>□</sup>
	%	%	
<b>Time period</b> 1990-1999 2000-2003	60.3 39.7	59.5 40.5	n.s.
<b>Age (at index pregnancy)</b> < 35 ≥ 35	68.3 31.7	77.9 22.1	<b>0.001</b>
<b>Parity</b> 0 1 ≥ 2	50.4 39.3 10.3	48.0 31.6 20.4	n.s. Ref < <b>0.0001</b>
<b>Civil status (at registration)</b> Married or cohabiting Not married/cohabiting	96.6 3.4	90.7 9.3	<b>0.001</b>
<b>Multiple pregnancy</b>	2.7	2.0	n.s.
<b>Hypertensive disorders</b> Preeclampsia/ eclampsia Hypertension	7.3 4.6	4.4 4.1	<b>0.035</b> n.s.
<b>Diabetes</b> Diabetes type 1 or 2 Gestational diabetes All diabetes	0.0 0.4 0.4	0.5 1.3 1.8	n.s.
<b>Placental abruption</b>	0.8	0.9	n.s.
<b>Placenta previa</b>	0.8	0.6	n.s.
<b>Smoking (at first visit)</b> Non-smoker Smoker	91.6 8.4	85.1 14.9	<b>0.007</b>
<b>Small-for-gestational-age</b>	1.5	2.1	n.s.

## 6.3 The data

### 6.3.1 Sociodemographic and clinical variables

An advantage of using data from medical records, rather than from a registry like the MBR, is that all information of interest can be extracted and the data are easily validated by reviewing the

records. This ensures the quality of the data. The data retained in the MBR is forwarded from delivery institutions. Data regarding each delivery is transferred to a standardized form, often by the midwife handling the delivery, and then subsequently forwarded to the MBR. Some variables of interest might be registered incorrectly or are not registered at all in the database. Normally there is not an opportunity for the researchers to validate the data. Thus the information extracted from the database is dependent on the information that was recorded at the time of registration. This would in our study apply an information bias, differentiated between the cases and the facility-based controls, since the data are from different sources. Studies have demonstrated that data on pregnancy complications like hypertension and gestational diabetes are often underreported in databases like the MBR [242,243]. This would lead to an overestimation of the association between certain conditions and IUFD when comparing the cases with the facility based control group. We believe we have an example of this in our study, as when we compared the cases to the facility based controls, the OR's for the associations of hypertension, preeclampsia or gestational diabetes and IUFD were greater than when comparing the cases with the selected controls (Table 5.1, page 72, Model A and Model B respectively).

Another advantage of using data from medical records was information on additional variables, not provided by the MBR. Information of additional variables is valuable in assessing confounding and in detecting co-linearity and interaction. We had an example of this relating to the association of single civil status and IUFD. When we compared the cases with the selected controls and added smoking to the multiple logistic regression model, single civil status was no longer significantly associated with IUFD (Table 5.1, page 72, Model C). We looked further into this and found that single women smoked more often than women married or co-habiting. There was another example of this with respect to the association of hypertensive disorders (HD) and IUFD. We had information on SGA among the selected controls. When we added SGA to the

statistical model we detected an interaction between HD and SGA (Table 5.1, page 72 Model C). The risk of IUFD related to hypertensive disorders was mediated through SGA, and was low or moderate if not accompanied by SGA.

### ***Small-for-gestational-age (SGA)***

SGA is not synonymous with intrauterine growth restriction (IUGR), as some SGA-fetuses may be constitutionally small while some normal-sized fetuses might be originally large-for-gestational-age (LGA) that are growth restricted. The choice of what to use in studies often depends on what kind of data are available, but SGA is commonly used as an approximate for IUGR, as we have done in this study. For identification of SGA it would have been preferable with customized growth charts that take into account maternal height, weight, parity, ethnic origin and the baby's gender [129,244]. We used population based growth charts to identify SGA since we did not have information on ethnicity and the information on maternal height and weight was often missing. The use of population based growth charts is reported to underestimate IUGR among preterm births [128]. If this were to be true in our material and the prevalence of SGA thus underestimated, the risk estimates for the association of SGA and IUFD would have been even higher.

The time of fetal death in our material was set at the time of diagnosis of the IUFD. Other authors have used the time of delivery minus 2 days as the estimated gestational age of IUFD, since that length of time is considered the average delay from fetal death to spontaneous or induced delivery [48]. By our method it is possible that we have in some cases overestimated the gestational age for time of death, which could in turn result in an overestimation of SGA. But it is unlikely that the true time of death would have differed more than a few days on average which probably has not affected the diagnosis of SGA considerably.

### **6.3.2 Placenta histology**

We found placenta pathology coded as a COD or an AC among 68% of the stillbirths. This was not unexpected since all these pregnancies had the very adverse outcome of stillbirth. However, there is a possibility that the focus on the placental investigation and placental pathology lead to over-diagnosis of placental pathologies. If fewer had been assigned a placental COD it is reasonable to believe that a greater proportion would have had an unknown cause. It is reported in the literature that a thorough examination of the placenta generally generates higher rates of stillbirths with placental causes and reduction in unexplained stillbirths [51,53].

Histological examination of the placentas from the controls would have been desirable. But, this was a retrospective study, and in the study period placentas were sent to histological examination on indication only. The pregnancies of live-born infants were in fewer instances pathological and therefore few of these placentas were referred to histological examination. We did not evaluate the character of the placental findings among women with different causes or risk factors for IUFD since that was not the scope of this study. Reports from other authors suggest that placental pathological changes are often non-specific [190].

### **6.3.3 Classification**

The assigned cause of death is a matter of expert opinion of the one who codes. In our study the classification was carried out by one person only (the author), which might be considered a weakness. It is preferable that each case is classified by two individuals and agreement sought in cases of inconsistencies. The qualities of the CODAC classification system have been tested and reported recently. The inter-observer agreement was reported to be generally fair, with a mean kappa of 0.65 [51], but good, with a kappa of 0.82-0.94 when the coding rules were extensively followed [60]. The ease of use received the highest scores of the classification systems tested

[51]. L.B.H. is an experienced obstetrician and the placenta histological examinations were done by experienced pathologists so we believe the coding is not a source of great bias. The distribution in causal groups was in agreement with the distribution in six other high-income countries recently reported which supports our findings [27].

#### **6.3.4 Blood sampling and analysis**

About 5-7% of healthy pregnant women have been reported to have positive tests for APAs [245]. The prevalence of APAs depends on the definition of a positive test. It is recommended by international consensus to define the cut-off values by the 99<sup>th</sup> percentile of the control group [246,247]. Since by definition 1% of the controls will have a positive result for each test, the prevalence of APAs in the healthy population will be influenced by the number of tests performed. Thus, if six different tests are used, up to 6% of the control/normal population will be APA positive.

The results of the Free PS assay in our study were expressed as percentages of normality, with reference values two standard deviations below the mean of the control group (53.7%). The Haematological Research Laboratory at OUH Ullevål has defined the normal range of free PS to be 65-130%, and values under 65% are defined as deficient. PS concentrations vary depending on several factors like age, gender, acquired conditions and the use of oral hormonal contraceptive or replacement therapy, which can lead to misdiagnosis of PS deficiency [178-181]. A report from 2010 demonstrated that lowering the cut-off values for PS and using the mean value of the controls minus 2 SD increased the specificity of the assays [182], which is why we have chosen to do so in our study.

Collection of blood samples only at a single time point and a long time after the index pregnancy are limitations of our study. However, one of the rationales for repeated testing of

APAs is to avoid false positive tests due to transiently elevated APAs, which we believe is not a concern in the present study, when the samples were collected 3-18 years after the index pregnancy. Neither is there reason to believe that any transient APAs should be more prevalent among the cases than the controls at this time.

### **6.3.5 Questionnaire**

Information from the questionnaire has not been a source for main analyses in any of the four publications included in the present thesis. Information from the questionnaires has been used to investigate: 1) factors that might influence the results of blood samples as pregnancy, hormonal or anticoagulation therapy and 2) prevalence of other incidents that might have similar causes or risk factors as stillbirths, especially miscarriages/fetal deaths in the first or second trimester before 23 gestational weeks. Information reported in questionnaires is subjected to the memory of the participants and can be an origin for recall bias. Regarding information on obstetrical history it is possible that women who have experienced IUFD have had more focus on and recall better the details of their reproductive/obstetrical history. As an example they might have a better memory of early miscarriages. This could result in more pathology reported by the cases, a systematic bias, that would in return cause an overestimation of the risk for pathology among the cases compared to the controls.

## **7. Discussion of main findings**

### **7.1 Incidence of IUFD**

The incidence of IUFD in our material was 4.1/1000 deliveries. For comparison, the incidence  $\geq 28$  completed gestational weeks in our material was 3.3 per 1000 (95% CI 2.9-3.6). This is in range with the rates reported in other Nordic countries. The rates of stillbirth  $\geq 28$  weeks in Sweden, Finland and Denmark, were in the period 1996-2000 reported to be 3.5 per 1000 births, 2.5 per 1000, and 4.0 per 1000, respectively [248] and in 2008 the rates were 2.7 per 1000, 2.0 per 1000 and 2.2 per 1000, respectively [21]. According to The Norwegian Medical Birth Registry the mean incidence of IUFD  $\geq 23$  weeks in Norway in the period 1990-2003 was 5.6 per 1000, but there was reported a decline in the rates from 6.8 per 1000 in 1990 to 4.2 per 1000 in 2003 [249]. We could not demonstrate this decline in incidence in our material. Neither could we confirm the finding of increasing incidence of IUFD as reported from England, Wales and Northern Ireland in the early 2000s [250], although we found a slight, but non-significant, increase in the incidence of stillbirth in the period 2000-2003 compared to the period 1990-1999. The difference in the stillbirth rates in our material and that reported in Norway in general could be explained by variations in stillbirth rates between different areas possibly caused by differences in the composition of the obstetric population or by different access to medical care. Norway is a large country with scattered inhabitation and long distances to medical facilities in some areas.

The literature is consistent about the declining incidence of stillbirths from the 1960's to the 1980's, after which the rates have been fairly constant. The decrease in incidence has been observed for stillbirth of almost all causes, although it has been more prominent in some groups like intrapartum deaths and deaths caused by iso-immunisation (Rhesus incompatibility), but

minimal in other groups, like deaths of infectious causes. In the same period there was a decrease in the absolute rates of unexplained stillbirths but their proportion of all stillbirths was the same in the 1980's as in the 1960's [38,47].

The obstetrical population is changing and compared to women giving birth in the 1960s, today the mothers are more likely to be older, nulliparous, unmarried, overweight, have hypertension or diabetes during pregnancy and to have had previous abortions [75]. In many Western countries the obstetric population is also changing due to immigration and now comprises a larger proportion of women with a minority background. Several studies have found the risk of stillbirth to vary considerably according to ethnicity [76,90,91,98,100,101]. All these factors can influence the stillbirth rates [25].

The stillbirth rate differed between the 2 hospitals. This may be related to the level of care of the hospitals. OUS is a regional and referral hospital for a large catchment area. AHUS is a county hospital and has a slightly lower level of service regarding neonatal intensive care and according to protocol the most premature pregnancies (< 26 weeks) are referred to OUS. There was no difference in the rates between the hospitals when stillbirths before 27 weeks were excluded. Our results are consistent with the findings of a Swedish study on a catchment area-based analysis of stillbirths and neonatal deaths. They found increasing ORs for mortality with increasing level of care of the delivery hospital [251]. Differences in the obstetric population of the two hospitals, according to ethnicity, could also have contributed to this difference. However, we did not have data on ethnicity since that information is not registered in the antenatal or hospital records and could thus not explore that further.

## **7.2 Risk factors**

A recently published meta-analysis found the following factors to be the most important risk



factors in high-income countries: maternal weight, maternal smoking, maternal age, primiparity, SGA, placental abruption and pre-existing maternal diabetes or hypertension, some of them potentially modifiable [34]. The factors that contributed most to the stillbirth risk at a population level, the population attributable risk (PAR), were found to be SGA <10<sup>th</sup> centile (PAR 23.3%), placental abruption (PAR 15.2%) and primiparity (PAR 14.3-15.3%) [34].

### **Maternal age**

In our material advanced maternal age was a significant risk factor for stillbirth in the multivariate analysis, when compared with facility-based controls. Most studies in the last two decades, both facility- and population-based, report advanced maternal age, in a variable magnitude, to be a risk factor for stillbirth [23,49,75-78,81,90]. A recent meta-analysis of Flenady et al. found maternal age of more than 35 years to be associated with a 65% increase in the odds of stillbirth (OR 1.65, 95% CI 1.61-1.71), but increasing with age over 40 (OR 2.29; 95% CI 1.54-3.41) [34]. One proposed explanation is failure of the uterine vasculature in older women to adapt sufficiently to the increased hemodynamic demands of pregnancy [104]. Authors who have studied the association between fetal growth restriction, advanced maternal age and stillbirth do not agree with this theory, since stillborn fetuses of older women have not been found to be more growth restricted than fetuses of younger women [48,252]. The maternal age-related risk of stillbirth is important since the obstetrical population is changing in developed countries and the number of births to women 35 years and older is increasing.

### **Parity**

We did not find the risk of stillbirth to differ by parity in the multivariate analysis. Some reports have shown increased risk among multiparous ( $\geq 3$  births) and/ or nulliparous women [75,77,78].

## **Smoking**

Smoking was an independent, significant risk factor for IUFD. When smoking was added to the statistical model, being single (neither married nor cohabiting) was no longer a significant risk factor for IUFD. This was in concordance with single women more often being smokers. The association between smoking and fetal death has been documented in several studies and placental pathology is suggested as a proposed pathway [24,78]. A recent meta-analysis of studies in high-income countries found a 36% increased odds of stillbirth associated with smoking (OR 1.36; 95% CI 1.27-1.46) and PAR of 4-7%, but higher in groups where smoking was more prevalent [34]. Raymond et al. demonstrated that in pregnancies of smoking women, with no known placental pathology/complications, there was not an increased risk of stillbirth [78]. They found this by excluding from analysis women with IUGR, placental abruption or placenta previa. Our results do not quite agree with this as we found smoking to be a risk factor of equal size in all causal groups as described in Paper IV. Other authors have documented an association between smoking and IUGR, where smoking more than 10 cigarettes per day was a strong risk factor for unexplained stillbirths among cases with IUGR [48]. We assessed the risk associated with smoking by smoking habits reported at first antenatal visit. Studies suggest that women who reduce or quit smoking in the first trimester have a comparable risk of stillbirth as non-smokers [253]. This emphasizes the importance of awareness of the risk associated with smoking and anti-smoking campaigns in early pregnancy.

## **Gestational age**

The conditional probability for IUFD in our material was unchanged through weeks 24–35, thereafter increasing continuously with advancing gestational age. Similar results have been reported elsewhere, even though the rate of stillbirths per 1000 deliveries decreases with advanced gestational age [57,77,78,115]. The trend in our material is consistent with a Scottish

study which examined the estimated probabilities of stillbirth from 37 gestational weeks and onward [114]. But despite this trend of progressive stillbirth risk we did not find the risk in gestational weeks 41 or 42 weeks to be significantly greater than the risk in week 40. Other authors have described similar findings [55,78]. The gestational-age related risk of stillbirth has been found to increase with advanced maternal age [77,78] and Hilder et al. observed that the risk for IUFD in post-term pregnancies was more evident among nulliparous women, particularly after 42 weeks [254].

### **Twin pregnancies**

Twin pregnancies, especially of monozygotic twins, are characterized by an increased incidence of fetal and maternal complications often related to placental pathologies, both common complications like hypertensive disorders, placental abruption and IUGR and complications unique to twin pregnancies as twin-twin transfusion syndrome [36,255].

### **Placental abruption**

We found the OR of the association of placental abruption and IUFD to be 15.4-22.0. Placental abruption is a leading cause of stillbirth, and reported to cause 15% of stillbirths in a recent analysis of causes of stillbirth in high-income countries [27], which was in agreement with our results of 16%. However placental abruption does not always cause stillbirth, although abruption involving 50% or more of the placenta frequently does [126]. A recent analysis reported the PAR of stillbirth related to placental abruption to be 15%, despite the low prevalence of abruption [34]. It appears that, in the vast majority of cases, abruption is the end result of a chronic process, a consequence of pathological placentation [126].

### **SGA**

We identified a greatly increased risk of IUFD related to SGA, and the risk related to

hypertensive disorders was mediated through SGA. Fetal growth restriction is reported to be the single largest category of conditions associated with IUFD (43%) [50,53]. Rather than being a diagnosis unto itself, SGA is a sign of a variety of other conditions that may lead to fetal death. Our result of SGA being almost equally prevalent in all the causal groups is a confirmation of that. Poor fetal growth, without other environmental causes, is assumed to indicate poor function of the placenta, but whether IUGR is a marker of placental insufficiency or causally associated with the mechanism of death is unclear [38]. SGA is also associated with congenital malformations and multiple pregnancies. Frequently though, none of these conditions are present in SGA pregnancies, as can be reflected by the large proportion of SGA-fetuses reported among unexplained IUFDs [48]. In prevention of stillbirth the correct diagnosis of IUGR is of great importance. In Northern Ireland, in the Confidential Inquiry into Stillbirth and Infant Death, it was concluded with that the most common error in antenatal care was failure to adequately diagnose and manage fetal growth restriction [224]. Evidence is lacking for the benefit of antenatal testing, although theoretically some methods could be adequate, like fundal height measurements [256], customized growth charts [257] and counting of fetal movements [258]. Doppler ultrasonography of the umbilical arteries has been demonstrated beneficial in reducing stillbirths among women in high-risk pregnancies with preeclampsia and suspected IUGR [259] and assessment of fetal growth by ultrasound has been demonstrated to improve outcomes in IUGR pregnancies [225].

### **Hypertensive disorders**

In our material, women with isolated hypertension (HT) had a greater risk of stillbirth than women with established preeclampsia (PE) and in fact when the cases were compared with the selected controls PE was not associated with stillbirth, unless in combination with SGA. We found that the major risk of IUFD associated with hypertensive disorders (HD) was mediated

through SGA. HD without SGA were only mildly associated with IUFD. This might be explained by HD and SGA being a symptom of the same condition, placenta insufficiency, with SGA representing a more serious form [126].

The difference in the risk of stillbirth associated with HT and PE can possibly be explained by that women with PE get better surveillance and induction of labor, when indicated, even in the preterm period, thus avoiding stillbirth, while women with HT only do not get adequate attention. In one study where the authors found chronic hypertension, but not gestational hypertension, to be associated with stillbirth it was presumed that the women with gestational hypertension received better surveillance and earlier delivery when indicated [133].

The pathogenesis of pregnancy related HD is assumed to be through placental pathology [131], therefore it was not unexpected to find HT as a risk factor in the group of placental causes of death. But HT was also found to be a risk factor in the group of unknown causes which could be explained by insufficient information to place them in the category of placental causes or that the placenta pathologies detected were subtle and not regarded as the major cause of death.

Other authors have reported either no [47,90] or a very modest association between hypertension and stillbirth [75,76,90], but comparison across studies is difficult because SGA is usually not adjusted for [76]. A recent report found pre-existing hypertension associated with a 2.6 times increase in the odds of stillbirth with PAR of 7-14% [34]. Preeclampsia was found to be associated with a more moderate risk, but still a 60% increase in the risk of stillbirth, with PAR of 3% because of the low prevalence [34].

### **Diabetes mellitus**

We found women with diabetes (pre-gestational and gestational) to have a three- to five-fold risk of stillbirth. This is in agreement with several other studies [81,146,148]. Stephansson et al. reported in a Swedish population a 14-fold increased risk of stillbirth associated with diabetes

mellitus but no association with gestational diabetes [81] and a British study found 5-fold increased rates of antepartum stillbirths among women with diabetes type 1 or 2 [148]. A recent meta-analysis also concluded with an association between pre-existing (OR 2.9; 95% CI 2.05-4.09), but not gestational diabetes [34]. Pre-existing diabetes mellitus is still one of the maternal medical disorders most strongly associated with stillbirth, although it does not contribute much to stillbirth risk at a population level (PAR 3-5%) [34]. However, reports from referral centers suggest that with optimal management the risk of perinatal death associated with diabetes is only marginally above that of the general population [75].

Diabetes was a significant risk factor for stillbirths of placental and unknown COD, but the OR in the unknown group was double that of the placenta group. The exact mechanism of fetal death in diabetes mellitus is unknown, but alterations in fetal carbohydrate metabolism and uteroplacental insufficiency secondary to vascular disease are possible explanations [260].

In 1999 women of non-Western origin comprised approximately 25% of the pregnant population in Oslo, the largest group from Pakistan [261]. These women have higher rates of diabetes as well as stillbirth [101,148]. One can speculate that some of the risk attributed to diabetes in our material actually relates to ethnicity, but since we did not have information on ethnicity, we could not correct for that in the multivariate analysis. However, a Swedish study found diabetes a significant risk factor for perinatal mortality even when adjusting for maternal origin [262].

### **Thyroid disorders**

We found thyroid disorders to be associated with stillbirth. Both hypo- and hyperthyroidism are reported to be associated with an increased risk of IUFD [46]. Fetal thyrotoxicosis may be the cause of death in some instances [263] and a two-fold increase in the risk of stillbirth is reported for women with hypothyroidism [264]. Hypothyroidism appears to be associated with increased

risk of hypertensive disorders [265]. Adequate thyroxin replacement improves the likelihood of a successful pregnancy.

There are few reports comparing risk factors according to cause. Frøen et al. evaluated risk factors among women with unexplained and explained stillbirths. The only significant difference he found was that women with explained stillbirth had more often glycosuria [1]. But he did also investigate variations in risk factors for unexplained fetal death in pregnancies with and without IUGR and found that among women with IUGR-pregnancies smoking, maternal overweight and low education were associated with unexplained stillbirth, while in pregnancies without IUGR, smoking was not a risk factor, but maternal age  $\geq 35$ , overweight, and low level of education were [48].

### **7.3 Thrombophilic risk factors**

Different pathways of the pathogenesis of thrombophilia associated pregnancy complications are constantly being investigated. Accumulating data are suggesting that an abnormal or exaggerated hemostatic response to pregnancy might be a potential cause. Several studies have demonstrated that some women with recurrent pregnancy loss are in a prothrombotic state when non-pregnant [266] suggesting that the further hypercoagulable state of pregnancy places them at risk of fetal loss and even a systematic thrombotic event. But other mechanisms of pathogenesis are being proposed and investigated. There are reports of that women with recurrent pregnancy loss are in a chronic state of endothelial stimulation associated with activation of the coagulation system [267]. Elevated levels of circulating procoagulant microparticles have also been described in the circulation of women with both early and late miscarriages, directly affecting the coagulation cascade in addition to possible pro-inflammatory and/or pro-apoptotic action disturbing successful implantation and subsequent fetal growth [268].

Few studies investigating the association of thrombophilia and stillbirth consider the influence of confounders. Kist et al. investigated the effect of confounders in a meta-analysis [269] and found the association of FV<sub>Leiden</sub> and several pregnancy complications to be confounded by ethnicity, genetic testing only and severity of disease. As an example, they found the association between FV<sub>Leiden</sub> and IUFD to be stronger in 2<sup>nd</sup> and 3<sup>rd</sup> trimester fetal losses rather than in 1<sup>st</sup> trimester recurrent fetal loss.

### **7.3.1 Inherited thrombophilia**

#### *Factor V Leiden*

We did not find a significant association between FV<sub>Leiden</sub> and stillbirth in general, but the prevalence was double among women with placental or unknown COD compared to the controls, although this did not reach statistical significance, probably due to small sample size. This finding could support a placental nature of the association. Case-control studies have variably demonstrated this association. A meta-analysis of prospective cohort studies in 2010 reported on PMPC [270]. Seven studies investigating the association of FV<sub>Leiden</sub> and pregnancy loss in all trimesters were included. For the association of FV<sub>Leiden</sub> and pregnancy loss they found a pooled OR of 1.52 (95% CI 1.06-2.19), but there were important inconsistencies in the definition of the outcomes and statistical heterogeneity across studies. When two studies including either spontaneous abortions or stillbirths only were excluded the association between FV<sub>Leiden</sub> and pregnancy loss was not significant (OR 1.34; 95% CI 0.9-1.98). Said et al. in his prospective study did find a positive association, but only six cases of stillbirth after 22 weeks were included in the study [271]. Meta-analyses including case-control studies have concluded with a mild to moderate association between FV<sub>Leiden</sub> and IUFD with ORs in the range 2.06-3.26 [5,6,272]. The included studies addressed IUFD after 20-27 weeks, but were of small sample size, comprising



3-67 cases, apart from one study that involved 232 cases [8]. Meta-analyses have concluded with that the association between FV<sub>Leiden</sub> and IUFD is stronger for women with more than one fetal loss [272] and when the fetal losses occur later in pregnancy [6,272]. More recently published case-control studies, not included in the meta-analyses, have not found FV<sub>Leiden</sub> to be a risk factor for IUFD [4,273]. Gonen et al. studied 37 women with stillbirths in gestational weeks 27-42 [4] and Pasquier et al. 58 women with unexplained pregnancy loss after 21 gestational weeks [4]. The divergent results across individual studies reporting on the association of FV<sub>Leiden</sub> and IUFD are probably caused by differences in study design, selection of cases and controls, definition of the gestational age at the time of IUFD, and sample size [274].

#### *The prothrombin gene polymorphism*

We found the prothrombin polymorphism to be significantly more prevalent among women with a history of IUFD compared to women with live births only. This association seems to be more consistently reported in the literature than the association of FV<sub>Leiden</sub> and stillbirth. A meta-analysis of case-control studies and several review articles have reported an association between the prothrombin polymorphism and late fetal loss with ORs in the range of 2.3-3.3 [6,157,275]. There are few prospective studies, with small sample sizes, that report on the association. Said et al. reported on 6 cases of IUFD after 20 gestational weeks in a cohort of 1707 women and did not find a significant association (OR 8.31; 95% CI 0.9-73) [271]. A meta-analysis of prospective cohort studies did not find an association between prothrombin polymorphism and pregnancy loss, but the sample size was found to be insufficient to detect important risk associations [270].

The finding of the prothrombin polymorphism to be significantly associated with stillbirths of placental causes would be expected since the main pathogenesis of stillbirth related to thrombophilia is assumed to be mediated through abnormalities in placental vasculature [186].

But even though a significant association was only found in the group of placental causes, the prevalence of the prothrombin polymorphism was similar in the group of other causes (8.2% and 6.1% respectively compared to 1.5% in the control group). Korteweg et al. did not find significant differences in the prevalence of thrombophilic defects when comparing women with a placental cause with women with a non-placental cause of stillbirth, but they did not compare women with different causes of stillbirth with live-birth controls [62].

#### *Antithrombin, Protein C- and protein S deficiency*

We did not find AT, PC- or PS deficiencies to be significantly associated with stillbirth. The reported associations of these deficiencies with IUFD are conflicting, which probably reflects the low prevalence of these conditions and thus the small numbers of deficient women investigated [6], which applies to our study as well. Thus most studies, including our study have not had sufficient power to demonstrate these associations. PS deficiency is usually found to be associated with IUFD, with high ORs, but not always significant because of few cases studied. A systematic review has demonstrated a significant OR of 20.1 (95% CI 3.7-109.2) based on 2 studies [6]. Korteweg et al. demonstrated recently that women with IUFD more often had significantly decreased levels of AT and PC during pregnancy compared with healthy pregnant women, although the levels remained within the normal range for non-pregnant women [276].

There is still great uncertainty regarding the association of inherited thrombophilia and stillbirth. But despite an increased relative risk of stillbirths and other adverse pregnancy outcomes (APO) associated with inherited thrombophilia, the absolute risk would remain modest because of the low prevalence of thrombophilias and the relatively low incidence of APO. The Risk and Economic Assessment of Thrombophilia Screening (TREATS) study [6] reviewed all prospective and retrospective studies concerning the association between thrombophilia and

venous thrombotic events (VTE) or APO and concluded with that universal thrombophilia screening among pregnant women was not supported by evidence. The study found selective screening based on previous VTE history to be more cost-effective. As long as there is uncertainty about the association between thrombophilia and stillbirth and while no good placebo-controlled intervention studies are available, prophylactic measures are to be thought of as experimental. A large placebo-controlled trial is being conducted at the moment, comparing low molecular weight heparin and placebo (saline) among pregnant thrombophilic women with a history of PMPC (<http://clinicaltrials.gov/ct2/show/NCT00967382>). Randomized controlled trials have been conducted among women with recurrent miscarriage of unknown origin and the use of anticoagulant therapy has not been proved beneficial [277,278].

### **7.3.2 Antiphospholipid antibodies**

In the present study, we found that women with a history of IUFD, as compared with women with live births only, were significantly more often positive for LA 3-18 years after the index pregnancy. The association was confined to women positive for LA in combination with other APAs. There is evidence that the presence of multiple APAs may increase the risk of thrombosis and severe pregnancy complications [279]. Therefore, patients with antiphospholipid antibody syndrome (APS) with multiple APA positivity are considered at higher risk for recurrence. Our study implies that multiple positivity is important, although firm conclusions cannot be made, due to the nature of the study and small sample size. Our results are in agreement with other recent studies that have demonstrated multiple positivity for APAs to be more frequently associated with pregnancy complications than single positivity [280,281].

We cannot exclude the possibility that some women have either turned negative or turned positive for APAs after the index pregnancy, but there is no reason to believe this to differ

between cases and controls. Remarkably little is known about the sustainability of APAs over time and we know of no studies that have investigated this among women with a history of IUFD. In one study, Erkan et al. found sustained positivity over time for approximately 75% of tests initially positive, but with a mean follow-up time of only 2.4, 3.5 and 1.0 years for LA, aCL, and anti- $\beta_2$  GP1, respectively [282].

Although there is possibly some increased relative risk of IUFD associated with APAs, the absolute risk for APA positive women without previous clinical events is low, and the probability of a successful pregnancy outcome is high. Thus, the screening for APAs in an unselected population of pregnant women is not recommended. The 8<sup>th</sup> Guidelines on Antithrombotic Therapy of the American College of Chest Physicians (ACCP) from 2008 recommend screening for APAs among women with a history of PMPC [195]. Low molecular weight heparin and low dose acetylsalicylic acid are currently only recommended for the prevention of recurrent pregnancy loss in women with APAs [195,232-234].

#### **7.4 Thrombophilia and placenta pathology**

Thrombotic lesions of the placenta are a common finding among women with stillbirth [283], as with other PMPC [186]. Of importance concerning the relationship between thrombophilia and placental pathology is whether coagulation abnormalities are underlying causes of abnormal placentation or adversely affect an already compromised placenta. No placental lesions have been demonstrated to be unique or specific for coagulopathy and the placental pathology for PMPC like IUGR, PE, placental abruption and stillbirth are very similar in patients with and without underlying maternal thrombophilia [190,284,285]. Abnormal uterine artery Doppler is in fact more predictive of villous infarcts and intervillous thrombosis than maternal thrombophilic disorders [286]. Studies have suggested that complications in late pregnancy may be determined

by impaired placental function already in the first 10 weeks after conception [122]. Presumably defective spiral artery transformation renders these vessels prone to thrombosis. Similar findings at delivery might simply represent a final common pathway for different underlying abnormalities.

## **7.5 Classification**

In the present study we found over half of all stillbirths to be caused by or associated with placental pathology (68.4%). A report from the Netherlands regarding classification of perinatal deaths by Korteweg et al. found 2/3 of the deaths to be caused by placental pathology, classified according to the Tulip classification [53,276]. They stated that classification systems without a group of placental causes or with only a minimal subdivision of this group were not useful in modern perinatal audit as loss of information would occur. Another recent analysis of perinatal deaths in high-income countries demonstrated that placental pathologies were causal or contributory in more than half of stillbirths [27]. Other reports have also found placental causes in up to 60% of the cases [69,72,287]. Rayburn et al. reported already in 1985 that most intrauterine deaths in the 3<sup>rd</sup> trimester were due to placental dysfunction, chronic or acute [287].

The risk of death in pregnancies with dysmature placenta has been shown to be 70-fold that of pregnancies with a normal placenta [288]. Placental dysfunction can be divided into chronic or acute dysfunction [288]. Early diagnosis of placental dysfunction is a major clinical challenge. For chronic dysfunction it is possible to detect IUGR, as the fetus does not grow according to its growth potential. No such markers are available for acute placental dysfunction.

Placental histological examination has received increasing attention in determining the causes of stillbirths as the findings can support or contradict the suggested diagnosis, or provide new clues [287,289] as has been pointed out by others [51,53,72]. Other main sources of

information in stillbirth investigation are the maternal and fetal health and history and the autopsy [51]. Availability of placental histological examination is reported to generate higher rates of stillbirths with placental causes and reduction in unexplained stillbirths [51,53]. But causality related to placental pathology can be unclear and avoiding over-interpretation of placental findings is thus important [255,290].

However, although placental failure is becoming a more recognized cause of stillbirth many classification systems do not address placental cause of stillbirth. Such classifications generate an excessive number of unexplained deaths otherwise allocated a placental cause in other classifications [53]. Newer systems like CODAC or the Tulip classification do recognize this group [60,62,69].

We reported 20% of IUFDs in our material with an unknown cause. A recent report of causes of stillbirth in 6 high-income countries, classified according to CODAC, reported 30% of the cases with an unknown cause and found the main reason for this a failure to perform adequate investigations as only 5% of stillbirths that underwent full assessment were unexplained [27]. The reported numbers of unexplained stillbirth in the literature ranges from 9 – 71% [49-51]. The proportion of unexplained stillbirths is dependent on both the sources of information available in addition to the classification system used. Classification systems capable of retaining important and detailed information have a lower proportion of unexplained deaths [62,69]. In a comparison with 5 other classification systems CODAC recieved the highest score regarding the ability to retain important information and for the ease of use. It had the lowest proportion of unexplained stillbirths and a fair inter-observer agreement [51].

The proportion of our cases distributed in the other causal groups was in agreement with recent reports by Flenady et al. [27] and Goldenberg et al. [42].

## 8. Conclusions

The incidence of IUFD was 4.1/1000 deliveries. Classified by the CODAC-classification half of all stillbirths had placental causes and 20% had an unknown cause that had placental associated conditions in 35.6% of the cases. SGA and placental abruption were found to be the strongest risk factors for IUFD. The risk of HD was mediated through SGA, and no or moderate risk was associated with HD if not accompanied by SGA. Other risk factors detected were, pre-pregnancy and gestational DM, thyroid disease, placenta previa, twin pregnancy, smoking, and advanced maternal age. These were of low prevalence and associated with low or moderate ORs and therefore probably of limited importance in further reducing the rates of stillbirths, although important in the prevention of IUFD in general. Smoking and SGA were significant risk factors across all causal groups. Women with IUFD of placental or unknown causes had many risk factors in common; twin pregnancy, hypertension and diabetes, which were not risk factors for IUFD of other causes. The risk estimates pointed in the same direction regardless of the control group, even though the absolute risk estimates were slightly different using different control groups.

Women with a history of IUFD after 22 gestational weeks were more often LA positive, 3-18 years after the incident, but the association seemed to be confined to women positive for other APAs in addition to LA. APAs as a composite variable were found to be associated with IUFD of other causes (than placental or unknown). There is however still great uncertainty related to the association of APAs and IUFD and the clinical importance is not easily predicated.

The prothrombin polymorphism was significantly associated with IUFD compared to women with live births only, but not FV<sub>Leiden</sub> or AT-, PC-, or PS deficiencies. When analyzed in separate causal groups the prothrombin polymorphism was a significant risk factor only among women with stillbirth of placental causes.

Stillbirth is a multifactorial disease including genetic, acquired and environmental determinants, which may interact on various levels. Many stillbirths in high-income countries are potentially preventable and preventive strategies that target risk factors are important in rate reduction. Prevention of stillbirths caused by placental insufficiency is dependent on the detection of women at risk and appropriate timing of birth, although planned early delivery needs to be weighed against the risk associated with intervening at a given gestational age. IUGR can be a sign of placental insufficiency, especially chronic placental dysfunction, and is the risk factor most frequently associated with stillbirth, thus the correct diagnosis of IUGR is of great importance. However, early diagnosis of placental dysfunction and IUGR are a major clinical challenge and no markers are available for acute placental dysfunction [288]. Screening of low-risk women has been demonstrated non-beneficial in detecting women at risk for complications like PE and/or IUGR [291,292]. A group of investigators has proposed the use of a “placental profile” as screening for the likelihood of PE, IUGR, placental abruption or IUFD among high risk women with medical and/or previous obstetric complications. They conducted a study estimating placental profile by 3 separate tests; maternal serum screening, second trimester uterine artery Doppler imaging, and placental morphologic condition. Women with all tests normal had significantly decreased risk of adverse pregnancy outcomes. If this profiling would accurately identify the subset of women at greatest risk for complications, this could be of advantage to both the patient and her physician, by providing reassurance to women with normal test results, and providing sufficient follow-up to the women at greatest risk for complications [293].

Recognition of the risks associated with medical disorders like diabetes and hypertension and implementation of guidelines that might improve management is of importance. Not all risk factors are avoidable, as for an example advanced maternal age. But awareness of the risks



attributed to such factors is important in choosing the appropriate follow-up regimen for these women. Theoretically awareness regarding the risk associated with advanced maternal age might also affect the women's choice of delaying childbearing.

Despite some increased relative risk of IUFD associated with thrombophilia, the absolute risk is low and thus the probability of a successful pregnancy outcome among women with thrombophilia high. There is no evidence to support universal screening of thrombophilia among pregnant women [294]. The 2008 guidelines from the American College of Chest Physicians (ACCP) on antithrombotic therapy and pregnancy recommend women with PMPC to be screened only for antiphospholipid antibodies (APAs), with a further investigation only if APAs are negative [195].



## **9. Future perspectives**

Further research is needed regarding appropriate measures to detect women/ pregnancies at risk for stillbirth, especially those at risk for placental dysfunction, which is a major contributor to stillbirth in high-income countries. The benefit of the interventions and cost-effective analysis, as on serial ultrasound examination for the detection of IUGR, should be investigated.

Benefit of other preventive strategies needs to be confirmed in studies, like weight management, efficacy of smoking cessation programs, improvements in antenatal care, use of interpreter in case of language barrier and risk reduction associated with induction of labor at different gestational ages.

Better understanding of the pathogenesis of IUFD among women with inheritable or acquired thrombophilia is needed in order to develop treatment options. It is possible that some still unknown thrombophilias could be involved in the pathogenesis of stillbirth. Recently low levels of protein Z [295,296] and reduced expression of annexin A5 [297,298], natural anticoagulant proteins, has been suggested to be associated with adverse pregnancy outcomes. This needs further investigation.

A large proportion of stillbirths are of placental causes. Further research regarding identification of clinical manifestations of placental pathology as well as focus on screening and interventions is important for the prevention of these deaths.



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## **Classification of Stillbirths by Cause of Death and Risk Factor Analysis - an Observational Case-control Study**

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**Short title:** Classification of stillbirths and risk factors.

## **Précis**

Classification of stillbirths by the Cause Of Death and Associated Conditions (CODAC)

classification system and socio-demographic, clinical and thrombophilic risk factors by cause.



## Abstract

**OBJECTIVES:** To classify stillbirths applying the Causes of Death and Associated Conditions (CODAC) - classification system and to investigate risk factors by cause.

**METHODS:** Observational case-control study of 377 women with stillbirths after 22 gestational weeks, and 1 215 controls with live births at two University hospitals in Oslo, Norway during 1990-2003. The stillbirths were classified according to CODAC based on relevant information from medical records and validated placenta histology. Socio-demographic, clinical and thrombophilic risk factors were estimated by causal groups.

**RESULTS:** A total of 190 (50.4%) women had placental and 73 (19.4%) unknown causes of stillbirth. In addition 68 (18%) cases with a non-placental or an unknown cause had placental associated conditions. Smoking and small for gestational age (SGS) were significant risk factors in all causal groups, while twin pregnancy, hypertension and diabetes were risk factors only for placental and unknown causes of stillbirth. Inherited thrombophilia combined and the F2 rs179963 polymorphism alone were significant risk factors for stillbirth of placental causes and antiphospholipid antibodies for stillbirth of other causes.

**CONCLUSIONS:** Over half of all stillbirths (68%) were caused by or associated with placental pathology. Risk factors differed somewhat according to cause, apart from smoking and SGA that were significant risk factors across all causal groups. Similar risk factors were detected among women with placental and unknown causes of stillbirth.

## INTRODUCTION

Classification of stillbirths is needed for the purpose of prevention, counselling and comparison of health care. Assigning a single cause of death can be challenging because of interaction between pathophysiological processes in the mother, placenta and fetus. Ideally classification systems for stillbirths should be able to capture both the direct cause of death and other clinical entities, as this can be important for deeper insight into the etiology. Suboptimal classification systems may lead to loss of information and a higher proportion of unexplained deaths. No single system is universally accepted, and this impedes comparison of stillbirth data. Data on risk factors by cause are limited.

The CODAC (Causes of Death and Associated Conditions) classification system for perinatal death is designed to retain information on the main cause of death as well as up to two associated conditions (1). It was developed by an international collaboration and has recently been evaluated and compared with 5 other classification systems (2). CODAC received the highest score regarding the ability to retain important information and for the ease of use. It had the lowest proportion of unexplained stillbirths and good inter-observer agreement (2).

Placental histology can support or contradict suggested diagnoses, or provide new clues and has received increasing attention in determining the cause of stillbirth. Many classification systems do not include placental causes of stillbirths, although most new classifications do (1,3,4). The aims of this study were to classify stillbirths according to CODAC and to study the association of risk factors by causal groups.

## MATERIAL AND METHODS

The present study was a part of a larger hospital-based case-control study; the Venous Thromboembolism In Pregnancy (VIP) study, and was registered as a clinical observational study at [www.clinicaltrials.gov](http://www.clinicaltrials.gov), with registration number NCT00856076 (5,6). Data on epidemiological, clinical (7) and biochemical risk factors for stillbirth (8,9) have been published.

All women with a diagnosis of stillbirth, from January 1<sup>st</sup> 1990 through December 31<sup>st</sup> 2003, at Oslo University Hospital Ullevål (OUH), Oslo, and Akershus University Hospital (AHUS), Nordbyhagen, both Norway, were identified retrospectively by a search for selected codes of the WHO International Classification of Diseases (ICD) versions 9 or 10, using the patient administrative system of the respective hospital. We identified 434 possible cases of stillbirth, defined as intrauterine fetal death in singleton or duplex pregnancies after 22 completed gestational weeks or birthweight  $\geq 500$  g. After reviewing the medical records, we excluded 49 cases wrongly diagnosed and eight with non-retrievable records, leaving 377 women with a verified diagnosis of stillbirth. The expected date of delivery was estimated by routine ultrasound examination at 16 to 18 gestational weeks for 73% of the women. In the absence of ultrasound data, gestational age was determined by the first day of the last menstrual period. The time of fetal death was determined by the gestational age at diagnosis. 1 229 women delivering at OUH in the study period were selected for the control group. After exclusion of 5 women under the age of 16 or over 44 years (none of the cases were in these age groups) and 9 women with stillbirth, the control group comprised 1 215 women with live singleton or duplex births at OUH in the study period.

The cases and controls were identified at the participating hospitals, medical records retrieved, and reviewed for validation of the diagnosis of stillbirth and other relevant

information. Individual data were transferred to a case-report-form which comprised data on demographics, general health, obstetrical history, details of the index pregnancy and delivery, post-mortem examination of the infant, and laboratory data including histological examination of the placenta. The case-report-forms were scanned, consistency analysis run and invalid data entries corrected after a review of relevant medical records. Risk factors assessed were maternal age, parity, marital status, assisted reproductive therapy, smoking habits, twin pregnancy, thyroid disease, pre-existing diabetes mellitus, gestational diabetes, hypertensive disorders, small-for-gestational-age (SGA), placental abruption and placenta previa. Validated population-based growth charts were used to determine whether the fetus was SGA, which was defined as birthweight below the 2.5<sup>th</sup> percentile for gestational age.

In 2006-2008 the cases and controls were invited to participate in the study to answer a questionnaire and to donate a blood sample. Women, who had emigrated, were foreign citizens, dead or had an invalid or unknown address, were excluded, and two additional cases had been identified. A letter of invitation was received by 346 cases of which 105 agreed to participate. The controls received first an invitation to participate in the thrombosis part of the VIP study (10,11), in which 353 agreed to participate. After exclusions, including 9 women with a history of stillbirth, 326 controls with live births received an invitation for this part of the VIP-study, and 262 agreed to participate (Figure 1).

Lupus anticoagulant (LA) was analysed with validated in-house lupus ratio tests (12), based on both activated partial thromboplastin time and Russell's viper venom time. Anticardiolipin (aCL) IgM and IgG antibodies were analysed with in-house ELISA tests and anti- $\beta_2$  glycoprotein 1 (anti- $\beta_2$ GP1) IgG and IgM antibodies with commercial ELISA kits (QUANTALite<sup>TM</sup>  $\beta_2$ GP1, INOVA Diagnostics Inc., San Diego, USA). The cut-off values for a

positive test were defined by the 99<sup>th</sup> percentile of the values of the control group. Antithrombin and protein C activities and free protein S were determined using commercial kits from Instrumentation Laboratory Inc, Bedford, MA, USA (Coamatic® Antithrombin, Coamatic® Protein C and HemosIL<sup>TM</sup> Free Protein S reagent kits, respectively). Women with Antithrombin activity < 80%, Protein C activity < 70% and Protein S two standard deviations below the mean of the controls were defined as deficient. The *F5 rs6025* (factor V Leiden) and the *F2 rs179963* (prothrombin gene G20210A) polymorphisms were detected with commercial detection kits (Roche Diagnostics, Basel, Switzerland). Two women reported use of warfarin medication at time of blood sampling and were therefore excluded from analyses of inherited thrombophilia.

The results of the original histological examinations of the placentas were reviewed. The placentas had originally been evaluated by general pathologists, but no standardized protocols for macro- and microscopic evaluation or sampling of placental tissue were in use in the study period. In most cases sections had been sampled from the umbilical cord, the membranes and minimum two sections from placental tissue from areas with and without focal parenchymal pathology. The tissue sections had routinely been fixed in formalin, processed and embedded in paraffin blocks, and 3.5 µm sections stained with Hematoxylin-Eosin.

For the purpose of a more accurate classification of causality, the original placenta specimens were retrieved and reassessed, when available. Placental tissue from 268 stillbirths were reassessed by two experienced pathologists (G.T. and B.R.) with special interest in placental pathology, blinded to the clinical details of the stillbirth. Cases with microscopic signs of acute or chronic villitis and/or intervillitis were immunostained with a standard panel of antibodies to T-cell- and histiocytic markers. When the placenta specimens were not available for reassessment the original histological descriptions (n=99) were used. Histological

descriptions of the placenta were available for 367/377 (97.3%) of the cases. Placenta information from controls was very limited because histological examination of the placenta is rarely carried out among normal birth giving women.

CODAC is a classification system for perinatal deaths, recently developed by an international collaborative group of investigators (1). In order to classify the case in CODAC, only minimum of information is mandatory but more detailed information usually generates a more accurate classification. The main focus is the “cause of death” (COD), with the possibility of coding for two additional “associated conditions” (ACs) to obtain more detailed information. For each case, CODAC thus allows up to three codes with three digits each (123 123 123), although only one code (123) is necessary. The first (or single) code represents the main COD. The second code can represent a secondary COD (if the first is not thought to be sufficient to fulfill the criteria of a solitary COD) or an AC, and the third code represents an AC. The first digit in each code represents “Level I” or the main categories of the COD or the AC (Digital supplement tables 1 and 2). The second and third digits, in each code, represent Levels II and III, each level representing more detailed information. Each Level I category are comprised of several Level II categories which in turn are comprised of several Level III categories. Ten coding rules have been defined for CODAC (1). To be a COD the condition should be expected to be mortal in a significant proportion of cases (5%) and to be an AC the condition should contribute significantly in explaining the circumstances of death. The system includes ten main groups (Level I), 94 sub-groups (Level II) and 577 sub-sub-groups (Level III). Two of the main groups were not relevant for coding in this study (group 9: termination and group 1: neonatal death). When all available information on each case had been reviewed it was assigned the most

appropriate code(s) according to the CODAC classification system by one of the authors (L.B.H.).

For detection of potential variations in risk factors according to cause, risk factors were analysed by comparing prevalences in different causal groups with prevalences in the control group. The eight actual main causal groups were analysed in three groups: placental causes (N=190), unknown causes (N=73) and other causes (the remaining six causal groups combined in one) (N=114). Risk factors were analysed by chi-squared tests or Fisher's exact tests and multiple logistic regression analysis. Missing values for sociodemographic and clinical variables were denoted the reference group. Variables included in the multivariate models were chosen based on the significance of each variable in the univariate analyses ( $p$ -value  $< 0.15$ ). The results are presented as percentages, odds' ratios (OR) and adjusted ORs (aOR) with 95% confidence intervals (CI). Significance level was set at  $p < 0.05$ . Interactions between significant factors were tested at a 95% significance level ( $p < 0.05$ ). All data was analyzed using the Statistical Package for Social Science version 16.0 (SPSS Inc, Chicago, IL, USA).

The Regional Committee for Medical Research Ethics, Region East, Norway, approved the study. Authorization for the use of information from medical records for research purposes was obtained from the Norwegian Ministry of Health and Social Affairs. The Norwegian Data Inspectorate approved the use of data comprising sensitive personal health information, and the merging of clinical and register-data by using the unique 11-digit personal identification number given to all Norwegian citizens at birth or immigration. All participants that donated a blood sample signed a written informed consent form.

## RESULTS

Women with stillbirth were more often single, cigarette smokers and pregnant with twins than the controls. They also had higher frequencies of medical disorders, including thyroid disease, diabetes and hypertension, and more often pregnancy related complications, such as preeclampsia, placental abruption and infants small-for-gestational-age (SGA) (Table 1).

The main COD (Level I) according to the CODAC classification system is displayed in table 2. Placental causes were the most frequent COD, accounting for 50.4% of all stillbirths, whereas unknown COD was second in line comprising 19.4% of the cases (Table 2). Subclassification (Level II) revealed that two-thirds (67.4%) of the placental causes were due to placental abruption/retroplacental hematoma or infarctions/thrombi. These two entities were responsible for 34% of all stillbirths and placental abruption/retroplacental hematoma alone was the cause of 16% of all stillbirths (Digital supplement table 3).

An AC was coded for 273/377 (72%) of all cases. The most frequent ACs were; placental in 92/377 (24%), cord pathologies in 51/377 (14%) and perinatal conditions in 55/377 (15 %). Among the cases classified as having an unknown COD, 59/73 (81%) had ACs; 26 (36%) a placental condition, 10 (14%) a cord condition, 10 (14%) a perinatal condition, 5 (7%) a maternal condition and 5 (7%) an associated maternal condition. A placental AC was assigned to 68 (18%) of the cases with a non-placental COD. Thus 258/377 (68%) of all cases had either a placental COD or placental ACs.

Prevalences of demographic and clinical risk factors among women with stillbirths and the controls are displayed in table 1 and the aORs of the risk factor analyses in table 3. Smoking and SGA were significant risk factors in all causal groups. The other significant risk factors differed by causal group. Twin pregnancy, hypertension and diabetes (pre-gestational and



gestational) were significant risk factors in both the placenta and unknown groups, but not among women with stillbirth of other causes. Placental abruption was a significant risk factor in the group of placental causes only, and single civil status only in the group of other causes. Thyroid diseases were associated with stillbirths of placental causes and of other causes.

The 105 cases who donated blood-samples for the biomarker study were equally distributed into the main COD groups as the whole study-population, 48% in the group of placental causes and 20% with an unknown cause. The prevalences of thrombophilia by different groups of COD are displayed in table 4 and the ORs for the association of thrombophilia and stillbirth in table 5. The *F2 rs179963* (prothrombin polymorphism) was more prevalent among women with placental COD and other causes, but statistically significant only in the group of placental causes (OR 5.7; 95% CI 1.4-23.8). The prevalence of *F5 rs6025* (factor V Leiden) was double among women with placenta or unknown COD compared to the controls, although this did not reach statistical significance. Inherited thrombophilia, a composite result, was significantly more prevalent among women with placental causes of stillbirth (OR 2.2; 95% CI 1.0-4.7). On the other hand being positive for any one of the antiphospholipid antibodies was associated with a history of stillbirth of other causes (OR 3.3; 95% CI 1.1-9.9). Similar proportions in all COD groups, 4.0-5.9%, of women were LA-positive as compared to 1.1% of the controls. However, the association did not reach significance in any separate causal group, although the ORs in the different groups were in the range 3.6 – 5.4 (Table 5).

## DISCUSSION

In an unselected population of women, we found that more than half of all stillbirths were caused by or associated with placental conditions (68.4%) similar to findings of two recently published studies (13,14). Korteweg et al. reported 64.9% of deaths to be caused by placental pathology and stated that classifications without or with minimal subdivision of a placental group were not useful in modern perinatal audit (14). Placental insufficiency probably originates early in pregnancy (15) and complications in late pregnancy may be determined by impaired placental function established already in the first 10 weeks (16). The distribution of women in other causal groups was in agreement with other recent reports (13,17).

The cause was unknown in 20% of the cases. A recent study of stillbirths in 6 high-income countries reported 30% with unknown causes and claimed the main reason to be inadequately investigated cases (13). The reported numbers of unexplained stillbirths ranges from 9-71% (2,18,19), dependent on the sources of information available and the classification system used (20,21). Systems capable of retaining detailed information have a lower proportion of unexplained deaths (3,4). A comparison of 6 classification systems found CODAC to have the highest score regarding the ability to retain important information (2). The importance of placental histology has been demonstrated (2,20,22) and may lead to lower proportions of unexplained stillbirths (20).

Smoking was equally frequent in all causal groups. Placenta pathology has been suggested as a pathway for the association of smoking and stillbirth (23,24), which is not compatible with our results. We registered smoking habits at first antenatal visit only, but smoking habits could have changed later in pregnancy which might have affected the results. Studies suggest that women who reduce or quit smoking in the first trimester have a comparable

risk of stillbirth as non-smokers (25). Stillborn infants were more often SGA compared to live-born unrelated to the cause of death. Fetal growth restriction is reported to be the factor most often associated with stillbirths and is probably a sign of a variety of conditions that may lead to fetal death (18,20). Whether it is a marker of placental insufficiency or causally associated with the mechanism of death is unclear (26).

Twin pregnancy, hypertension and diabetes were risk factors for stillbirths of both placental and unknown causes. Finding these risk factors in both groups can, in the case of unknown causes, suggest that data was inadequate to place them in the placenta group or that subtle placental pathologies found were more severe than assumed. Placenta pathology is assumed to be the origin of pregnancy related hypertensive disorders (27) and fetal and maternal complications are more frequent in twin pregnancies, especially monozygotic twins, in addition to unique complications as twin-twin transfusion syndrome (28,29). There was a strong association between diabetes and stillbirth of unknown causes. The mechanism of fetal death in diabetes is unknown, but alterations in fetal carbohydrate metabolism and uteroplacental insufficiency secondary to vascular disease are possible explanations (30).

Inherited thrombophilia combined and *F2 rs179963* alone were significantly associated with stillbirth of placental causes. This finding would be expected since the main pathogenesis of thrombophilia related stillbirth is assumed to be through abnormalities in placental vasculature (31). The prevalence of *F5 rs6025* was double among women with placental or unknown COD compared to the controls, although this did not reach statistical significance, probably due to small sample size. Korteweg et al. did not find significant differences in the prevalence of thrombophilic defects, but they compared women in different causal groups internally and not with live-birth controls (3). No specific placental lesions are found among women with

thrombophilia (32) and whether coagulation abnormalities are the causes of abnormal placentation or just exert an affect on a compromised placenta is not known. Similar findings at delivery might simply represent a final common pathway for different underlying abnormalities. Placental examinations from controls would have been desirable, but in the study period placentas were examined only on indication.

In CODAC the assigned cause of death is a matter of expert opinion. The cases were all classified by one author (L.B.H.) and possibly another coder would not have agreed in all cases. A kappa of 0.82-0.94 has been reported for CODAC when the coding rules are extensively followed (1). L.B.H. is an experienced obstetrician and the placenta histological examinations were performed by experienced pathologists (G.T. and B.R.), so the coding is not suspected to be a source of large bias. The distribution in causal groups was in agreement with the distribution in six other high-income countries (13), which supports our findings.

Early diagnosis of placental dysfunction is a major clinical challenge. Screening tests in low-risk groups have been demonstrated to be poor predictors of complications (33,34). However, “placental profile” has been proposed among women in high-risk groups, with somewhat promising results (35).

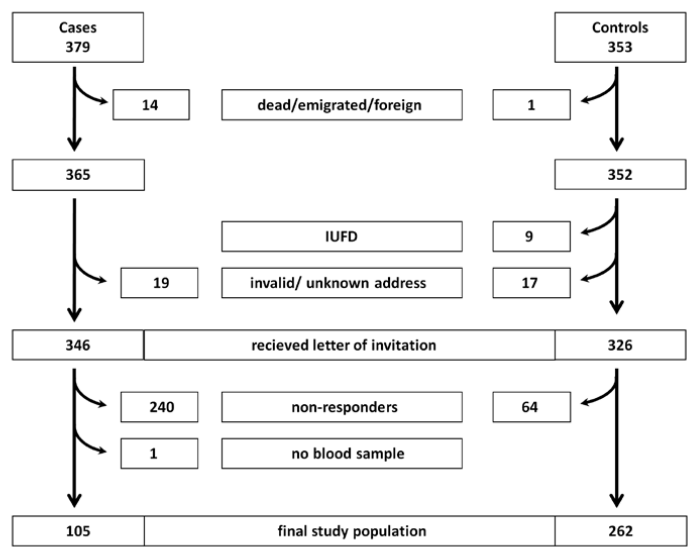
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**Figure 1.** Selection of study population for the biomarker study.





**Table 1.** Prevalence of demographic and clinical risk factors among women with IUFD, according to the main cause of death (COD) of the CODAC- classification.

<b>Variable</b>	<b>All cases N=377</b>	<b>COD Placenta N=190</b>	<b>COD Unknown N=73</b>	<b>COD Other N=114</b>	<b>Controls N=1 215</b>
	%	%	%	%	%
<b>Age (years)</b>					
≤29	44.6	42.6	49.3	44.7	43.6
30-39	49.9	52.1	45.2	49.1	50.5
≥40	5.6	5.3	5.5	6.1	5.8
<b>Parity</b>					
0	51.2	53.2	49.3	50.0	48.5
1	31.8	29.5	35.6	33.3	33.2
≥2	16.7	17.4	15.1	16.7	18.4
<b>Civil status</b>					
Married/Cohabitation	86.2	85.8	95.5	80.7	92.0
Not married/cohabiting	13.8	14.2	4.1	19.3	8.0
<b>Assisted reproduction</b>	1.9	2.6	2.7	0	1.7
<b>Twin pregnancy</b>	5.8	7.4	6.8	2.6	2.1
<b>Hypertensive disorders</b>	15.7	18.4	17.8	9.7	9.2
Preeclampsia/eclampsia	7.2	9.5	5.5	4.4	4.8
Hypertension	8.5	8.9	12.3	5.3	4.4
<b>Diabetes</b>					
Diabetes type 1 or 2	1.9	1.1	4.1	1.8	0.4
Gestational diabetes	1.6	1.1	4.1	0.9	1.0
<b>Placental abruption</b>	11.7	22.6	0	0.9	0.8
<b>Placenta previa</b>	1.3	2.1	0	0.9	0.7
<b>Smoking (at first visit)</b>	35.8	37.9	32.9	34.2	13.4
<b>Small-for-gestational age</b>	35.8	41.1	23.3	35.1	1.8
<b>Thyroid disease</b>	4.2	4.7	2.7	4.4	1.1

**Table 2.** Cause of death (COD) – level I – according to the CODAC- classification.

<b>CODAC</b>  <b>COD – level I</b>	<b>Cases</b>	
	<b>N=377</b>	
	<b>%</b>	<b>(n)</b>
<b>0-Infection</b>	12.2	(46)
<b>2-Intrapartum</b>	0.5	(2)
<b>3-Congenital anomaly</b>	6.1	(23)
<b>4-Fetal</b>	2.1	(8)
<b>5-Cord</b>	8.0	(30)
<b>6-Placenta</b>	50.4	(190)
<b>7-Maternal</b>	1.3	(5)
<b>8-Unknown</b>	19.4	(73)

**Table 3.** Risk factors for cases in different causal groups according to CODAC compared to controls. Adjusted odds ratios (aOR) with 95% confidence intervals (CI).

<b>COD</b> <b>Variable</b>	<b>Placenta</b> <b>N=190</b>	<b>Unknown</b> <b>N=73</b>	<b>Other</b> <b>N=114</b>	<b>All cases</b> <b>N=377</b>
	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
<b>Civil status</b>				
Not married/cohabiting	-	-	<b>2.1</b> <b>(1.1-3.9)</b>	-
<b>Twin pregnancy</b>	<b>3.6</b> <b>(1.3-9.4)</b>	<b>5.0</b> <b>(1.5-16.0)</b>	2.2 (0.6-8.0)	<b>3.0</b> <b>(1.4-6.3)</b>
<b>Hypertensive disorders</b>				
Preeclampsia/eclampsia	0.5 (0.2-1.1)	0.2 (0.06-1.0)	0.2 (0.07-0.8)	0.6 (0.3-1.2)
Hypertension	<b>2.2</b> <b>(1.0-4.8)</b>	<b>3.5</b> <b>(1.5-8.0)</b>	1.0 (0.3-2.9)	<b>1.9</b> <b>(1.1-3.4)</b>
<b>Diabetes</b>	<b>3.5</b> <b>(1.1-11.3)</b>	<b>8.7</b> <b>(3.0-25.4)</b>	2.9 (0.8-10.8)	<b>3.8</b> <b>(1.7-8.4)</b>
<b>Placental abruption</b>	<b>42.0</b> <b>(19.4-91.0)</b>	-	1.4 (0.2-11.5)	<b>16.1</b> <b>(7.6-33.9)</b>
<b>Smoking (at first visit)</b>	<b>2.5</b> <b>(1.6-3.9)</b>	<b>3.7</b> <b>(2.1-6.6)</b>	<b>2.5</b> <b>(1.5-4.1)</b>	<b>2.6</b> <b>(1.9-3.8)</b>
<b>Small-for-gestational age</b>	<b>47.9</b> <b>(26.6-86.1)</b>	<b>28.6</b> <b>(12.8-63.6)</b>	<b>38.6</b> <b>(19.9-74.8)</b>	<b>32.9</b> <b>(20.0-54.2)</b>
<b>Thyroid disease</b>	<b>5.5</b> <b>(2.0-15.6)</b>	3.8 (0.8-18.0)	<b>5.5</b> <b>(1.7-17.6)</b>	<b>4.8</b> <b>(2.1-11.0)</b>

Each variable adjusted for the all the other variables in logistic regression analysis.

**Table 4.** Prevalences of acquired and inherited thrombophilia by different main groups of COD.

<b>COD</b>  <b>Variable</b>	<b>All</b>  <b>N=105</b>  <b>*N = 103</b>  <b>% (n)</b>	<b>Placenta</b>  <b>N = 50</b>  <b>*N=49</b>  <b>% (n)</b>	<b>Unknown</b>  <b>N = 21</b>  <b>*N=21</b>  <b>% (n)</b>	<b>Other</b>  <b>N = 34</b>  <b>*N=33</b>  <b>% (n)</b>	<b>Controls</b>  <b>N =262</b>  <b>% (n)</b>
<b>*<sup>&amp;</sup>F5 6025</b>	10.7 (11)	14.3 (7)	14.3 (3)	3.0 (1)	7.6 (20)
<b>*<sup>#</sup>F2 rs1799963</b>	5.8 (6)	8.2 (4)	0	6.1 (2)	1.5 (4)
<b>*Antithrombin deficiency</b>	1.0 (1)	0	0	3.0 (1)	0
<b>*Protein C deficiency</b>	1.0 (1)	0	0	3.0 (1)	1.1 (3)
<b>*Protein S deficiency</b>	1.9 (2)	2.0 (1)	0	3.0 (1)	2.3 (6)
<b>*Any inherited thrombophilia</b>	18.4 (19)	22.4 (11)	14.3 (3)	15.2 (5)	11.8 (31)
<b>Lupus anticoagulant</b>	4.8 (5)	4.0 (2)	4.8 (1)	5.9 (2)	1.1 (3)
<b>Anti-cardiolipin antibodies</b>	3.8 (4)	2.0 (1)	4.8 (1)	5.9 (2)	2.3 (6)
<b>Anti-<math>\beta_2</math>-glycoprotein 1</b>	4.8 (5)	6.0 (3)	0	5.9 (2)	1.5 (4)
<b>Antiphospholipid antibodies</b>	9.5 (10)	6.0 (3)	9.5 (2)	14.7 (5)	5.0 (13)
<b>*Any thrombophilia</b>	27.2 (28)	28.6 (14)	23.8 (5)	27.3 (9)	16.4 (43)

\*Two women reported use of warfarin and were therefore excluded from all analyzes of inherited thrombophilias. <sup>&</sup>Also known as factor V Leiden; <sup>#</sup>also known as the prothrombin gene 20210 allele variation.

**Table 5.** Thrombophilic risk factors for IUFD according to COD compared to controls.

Odds ratios (OR) and 95% confidence intervals (CI).

Variable \ COD	All N = 105 *N=103	Placenta N = 50 *N=49	Unknown N = 21 *N=21	Other N = 34 *N=33
	OR 95% CI	OR 95% CI	OR 95% CI	OR 95% CI
* <sup>&amp;</sup> F5 6025	1.4 (0.7-3.1)	2.0 (0.8-5.1)	2.0 (0.5-7.4)	0.4 (0.05-2.9)
* <sup>#</sup> F2 rs1799963	<b>4.0</b> <b>(1.1-14.4)</b>	<b>5.7</b> <b>(1.4-23.8)</b>	-	4.2 (0.7-23.7)
*Antithrombin deficiency	-	-	-	-
*Protein C deficiency	0.8 (0.09-8.2)	-	-	2.7 (0.3-26.7)
*Protein S deficiency	0.8 (0.17-4.3)	0.9 (0.1-7.6)	-	1.3 (0.2-11.4)
*Any inherited thrombophilia	1.7 (0.9-3.1)	<b>2.2</b> <b>(1.0-4.7)</b>	1.2 (0.3-4.5)	1.3 (0.5-3.7)
Lupus anticoagulant	<b>4.3</b> <b>(1.0-18.4)</b>	3.6 (0.6-22.1)	4.3 (0.4-43.4)	5.4 (0.9-33.5)
Anticardiolipin antibodies	1.7 (0.5-6.1)	0.9 (0.1-7.4)	2.1 (0.2-18.6)	2.7 (0.5-13.8)
Anti- $\beta$ 2 glycoprotein 1	3.2 (0.9-12.3)	4.1 (0.9-19.0)	-	4.0 (0.7-22.9)
Any antiphospholipid antibodies	2.0 (0.9-4.8)	1.2 (0.3-4.5)	2.0 (0.4-9.6)	<b>3.3</b> <b>(1.1-9.9)</b>
Any thrombophilia	<b>1.9</b> <b>(1.1-3.3)</b>	<b>2.0</b> <b>(1.0-4.1)</b>	1.6 (0.6-4.6)	1.9 (0.8-4.4)

\*Two women reported use of warfarin and were therefore excluded from all analyses of inherited thrombophilias. <sup>&</sup>Also known as factor V Leiden; <sup>#</sup>also known as the prothrombin gene 20210 allele variation.

